
***DIELS-ALDER APPROACHES
TO ANTI-CANCER PRODRUGS***

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by

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***DIELS-ALDER APPROACHES
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ABSTRACT

This thesis concerns the design and synthesis of compounds relevant to a new strategy for the synthesis of anti-cancer prodrugs. The focus of this strategy is Diels-Alder chemistry leading to oxygen or sulfur-bridged polycyclic adducts. Bridge cleavage of such adducts can result in the formation of polycyclic, aromatic compounds, a structure characteristic of anti-cancer drugs which act via intercalation.

Part A describes the design of a model system for the synthesis of Diels-Alder adducts as prodrugs. A range of isobenzofurans, along with a range of fumaramide and *N*-aryl maleimide derivatives, were synthesised, and these were reacted together in Diels-Alder fashion. The resulting adducts were bridge cleaved.

Part B describes the introduction of functional groups into Diels-Alder adducts. The functional groups investigated included alkylating and hydrogen-bonding substituents, introduced via *N*-aryl maleimide based dienophiles. A range of isobenzothiophenes was also synthesised, and these were used to generate sulfur-bridged Diels-Alder adducts, with the resulting increase in stability proving advantageous in some instances. A variety of functionalised adducts and their bridge cleaved derivatives were prepared. The biological properties of some showed promise as leads for the development of anti-cancer compounds.

ABBREVIATIONS

DCC	1,3-Dicyclohexylcarbodiimide
DCU	<i>N,N</i> -Dicyclohexylurea
DMF	<i>N,N</i> -Dimethylformamide
DMSO	Methyl sulfoxide
DNA	Deoxyribonucleic acid
EI	Electron ionisation
Et	Ethyl
FAB	Fast atom bombardment
HOBt	1-Hydroxybenzotriazole
LDA	Lithium diisopropylamide
Me	Methyl
NMR	Nuclear magnetic resonance
Nu	Nucleophile
Ph	Phenyl
ppm	Parts per million
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran

Chapter 1

APPROACHES TO CANCER CHEMOTHERAPY

1.1 Introduction

This thesis concerns the design and synthesis of potential anti-cancer compounds, which may offer advantages over existing compounds. The introductory chapter describes cancer and the limitations of conventional forms of anti-cancer treatment. Novel approaches to potential anti-cancer prodrugs which selectively target tumour cells are discussed. DNA intercalation and its relevance to anti-cancer treatment is overviewed. This is followed by a description of the new class of potential prodrugs which are investigated in this thesis, along with the general strategy employed for their synthesis using Diels-Alder chemistry.

1.2 Cancer

Cancer accounts for about 1 in 5 deaths in Western countries.¹ The term cancer covers a wide range of diseases which are characterised by uncontrolled cell replication. Cell growth is regulated carefully in human and other mammalian cells, but mutations in DNA can result in a breakdown in this regulation. The body of cells formed by uncontrolled replication is termed a tumour. A tumour can be either benign or malignant. In its benign form, a tumour is localised and relatively non-dangerous to the host. A malignant tumour, however, invades surrounding healthy tissue and cells which break off from it can be carried around the body to set up secondary sites of cancerous growth. This latter condition, termed metastasis, is often fatal to the host.

Cancer can currently be treated in several ways, notably surgery, radiotherapy, and chemotherapy. Surgery involves the physical removal of the tumour before it has begun to metastasise. Radiotherapy involves the bombardment of the tumour with ionising radiation leading to cancer cell death. Chemotherapy involves using chemicals to induce cancer cell death. These treatments are often combined.

1.3 Cancer Chemotherapy

Drugs are currently used at some stage during the treatment of most cancer patients.² Chemotherapy is the treatment of choice for most metastatic cancers. Cure rates of 50% or greater have been achieved using combination chemotherapy for such cancers as *diffuse histiocytic lymphoma*, *Hodgkin's disease*, *acute lymphocytic leukemia*, *Wilm's tumour*, and *childhood sarcomas*. *Testicular carcinoma* has a cure rate of over 75% and, by using a combination of the drugs

methotrexate and actinomycin D, *gestational choriocarcinoma* has a cure rate greater than 90%.

The objective of cancer chemotherapy is to reduce the tumour cell population to zero. In practice however, this is extremely difficult to achieve. Frequently chemotherapy results in prolonging patients' lives rather than effecting permanent cures. This can be achieved by successfully inducing remission of the tumour, followed by regimens of maintenance therapy, which employs the prolonged use of moderately toxic drugs to curb cancer regrowth. With respect to solid tumours however, such maintenance therapy is rarely effective and hence seldom used.

Cancer chemotherapy is used effectively however, to prevent or delay tumour recurrence in patients who have had primary tumours surgically removed. Such patients are at a high risk of recurrence and chemotherapy regimens have successfully extended the survival of patients with cancers such as *breast cancer*, *colon cancer*, and *osteosarcoma*.

1.4 Limitations of Conventional Cancer Chemotherapy

There can be no question though that, in spite of their benefits, conventional cancer treatments have severe limitations. For example *bowel cancer* is the third most common cancer worldwide, and yet there is no effective chemotherapy available for this disease.³ Although it can be cured by surgery in its early stages, bowel cancer often goes undetected until intervention is no longer any use, and about half of all patients die.

Additionally, both cancer radiotherapy and chemotherapy are limited by severe side-effects. The major drawback with conventional cancer chemotherapy is that it is rarely cancer specific but is rather specific to rapidly dividing cells. Such

rapidly dividing cells are not limited to cancer but are also characteristic of normal human cells in the gut, skin epithelium, bone marrow, hair, and mouth mucosa. This is the basis of many common side-effects of cancer chemotherapy, such as vomiting, nausea, hair loss, skin irritation, and mucous membrane irritation. A long term side-effect may be bone marrow depression.

Other limitations of conventional cancer chemotherapy are drug resistance and the presence in solid tumours of a significant portion of slowly dividing cells which are unaffected by conventional drugs. Drug resistance occurs when a certain portion of the tumour is resistant to a particular drug. This resistant section of the tumour will not be killed by the drug and may result in a drug-resistant secondary tumour. To a certain extent the limitation of drug resistance can be overcome by using combination chemotherapy, where drugs with different mechanisms of action are combined. The presence of slowly dividing cells in solid tumours however presents a different challenge.

Many human cancers are slow growing solid tumours.⁴ This presents the challenge of finding and exploiting biochemical differences other than a high rate of cell division. One example of such a difference is hypoxia. Solid tumours are characterised by the presence of hypoxic, or oxygen-deprived, cells.^{5,6} As a solid tumour grows, the cells face a challenge in inducing the development of additional blood supply. As a consequence a significant proportion of the tumour cells are an appreciable distance from any blood vessels. At more than 150µm from a blood vessel the concentration of oxygen falls to the point where cells further away than this suffer oxygen deprivation. In fact such oxygen-starved, or hypoxic, cells often surround a region where cells have actually died from lack of oxygen. Hypoxia in tumours can also be caused by the spasmodic blocking of blood vessels due to the high fluid pressure present in tumours.

Unlike rapidly replicating cancer cells, often targeted by anti-cancer drugs, hypoxic cells are starved of oxygen and nutrients and hence very slow to replicate. This means they are not susceptible to most conventional anti-cancer drugs. It also gives them increased resistance to cancer radiotherapy, due to the need for molecular oxygen in the inactivation process. The added danger of hypoxic cancer cells however, is that once the rapidly replicating cancer cells around them have been killed off by anti-proliferative cancer drugs, the blood supply is eventually restored and the tumour is able to commence growing again.

1.5 Prodrug Approaches to the Treatment of Solid Tumours

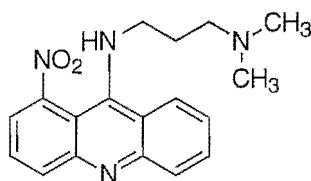
The characteristics of solid tumours, which present an obstacle to conventional cancer chemotherapy, can potentially be used to advantage in cancer treatment, as they differentiate the tumour from normal human cells. One approach to selectively target solid tumours is the use of prodrugs.

A prodrug is the precursor to a drug. It has lower toxicity than the drug itself, and is designed to be activated to the drug at the site of the disease, and only at that site, in this case the solid tumour. This means that it is a localised form of cancer treatment, and might have little effect on other parts of the body. As a prodrug is always moving down a concentration gradient, it has the additional feature of increased efficiency of delivery to the target site.

In order to be suitable as an anti-cancer treatment, a tumour cell selective prodrug must incorporate several key features. It must convert rapidly and selectively to the active drug in the conditions of a solid tumour. It must be of low or zero toxicity. It must be stable to normal metabolism. It must be able to effectively reach the target cells.

The activated drug itself should also incorporate several key features. It must be of high toxicity. It should have a relatively long half-life. It should be able to diffuse to neighbouring tumour cells.

Prodrugs can be activated by many methods. For example, some cells of a solid tumour can be targeted by exploiting their hypoxia. Since such cells are oxygen deficient, reduction can be used to activate the prodrug. Reductions which are reversible when oxygen is present in a cell may become irreversible under oxygen-deficient conditions. So the activating process best suited for this chemistry involves a normally reversible redox component, to avoid activation in normal cells. Nitracrine **1.1**, shown in Fig. 1.5.1, is one such hypoxia-selective prodrug.⁷



1.1

Fig. 1.5.1 Nitracrine

Nitracrine **1.1** is thought to be activated in oxygen-deficient conditions by reduction of the nitro group to an amino group. In this form the compound is potentially a potent DNA intercalating agent and also able to form cross links by nucleophilic attack of the amine group. When tested clinically, nitracrine was found to be extremely hypoxia-selective against cultured tumour cells. However *in vivo* its activity was not marked. This was thought to be due to its low diffusion rate, caused by high DNA binding affinity, and its metabolism in the body.

There are a range of possible ways of activating a prodrug selectively at a tumour site. This thesis documents synthetic chemistry pertinent to developing a class of prodrugs which could be activated to DNA intercalating agents. These methodologies can be developed to incorporate a variety of triggers activated by a range of biochemical processes. An overview of DNA intercalation and its relevance to anti-cancer therapy is therefore presented now.

1.6 Intercalators as Anti-cancer Agents

Intercalation involves the insertion of a chromophoric, or planar, part of a molecule between two stacked DNA base pairs.⁸ While the primary and secondary structure of the DNA remain completely intact in this process, the tertiary structure, or helix, is altered. The DNA helix is partially lengthened and as a consequence is also slightly unwound in relation to the original structure. Intercalation thus results in the average separation between two stacked base pairs essentially doubling, increasing from an original 3.4Å to approximately 7-8Å.

As would be expected, this alteration in DNA topology has a profound effect on its biochemistry. Enzymatic blockade and reading errors in the replication process disturb the matrix function considerably. There are a variety of mechanisms by which intercalators can act. For example intercalation can result in DNA damage via interference with the action of the enzyme topoisomerase II, which promotes DNA strand breakage and resealing. An alteration in the 3-dimensional structure of DNA can arrest the cycle of topoisomerase II action at the point of DNA cleavage. By this means, or by a variety of other processes, intercalation of the planar part of a molecule into DNA can result in DNA damage.

There are several forces which determine an intercalator's DNA binding affinity. These include favourable Van der Waal's Forces between the intercalating agent and the base pairs of DNA, along with favourable electrostatic and hydrogen bonding interactions between the substituents on the intercalating agent and the DNA.

In some instances it has been shown that there is a relationship between an intercalator's binding affinity to DNA and its *in vitro* as well as *in vivo* anti-cancer activity.⁹ For example, some potent intercalators appear to form stable ternary complexes with DNA and topoisomerase II, which interferes substantially with DNA resealing. In these cases the resulting breaks in the DNA have been directly correlated with cytotoxicity.¹⁰ However, due to the variety of mechanisms by which intercalators can act, anti-cancer activity is often only tenuously related to DNA binding strength.

The intercalating portion of an intercalator is generally made up of at least three coplanar, fused rings, which are often aromatic. For optimal intercalation, the planar portion of the molecule, also known as its chromophore, must possess a minimum surface area of 28\AA^2 . In practice this means 3 to 4 fused, coplanar rings. A prerequisite for intercalating agents with significant cytotoxic properties appears to be not just the presence of a coplanar system but also the presence of one or more substituents capable of interacting with DNA and affecting its biochemistry.

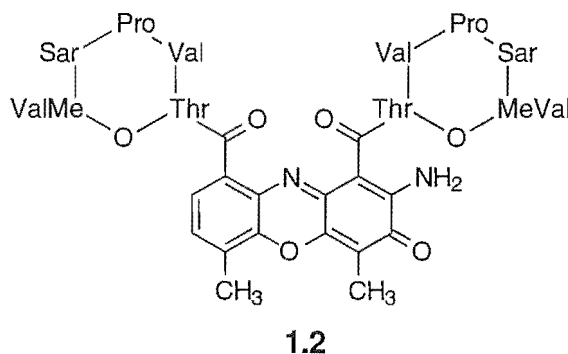


Fig. 1.6.1 Actinomycin D

One example of a non-topoisomerase II acting intercalator, which has potent anti-cancer properties, is actinomycin D **1.2**, which is shown in Fig. 1.6.1.^{11,12} Actinomycin D **1.2** is known to be a strong DNA-binding drug. It is a powerful inhibitor of RNA and protein synthesis.¹³ Muller and Crothers proposed a model for DNA intercalation of actinomycin D in which its chromophore lies intercalated between base pairs with the peptide lactone rings lying in the minor groove.¹⁴ When cyclic peptides such as these lie in the grooves of DNA they can affect the activity of polymerase enzymes. This model was based on spectroscopic and hydrodynamic data and has been backed up by x-ray crystallographic studies. Actinomycin D has been shown to accumulate in tissue by means of passive diffusion.¹⁵ As a consequence the efficiency of actinomycin D, as an anti-cancer drug, is dependent on the ability of the cell to accumulate the drug, and its activity varies for different tumours.^{16,17,18} Actinomycin D has proved itself a powerful cytotoxic drug for the treatment of *gestational choriocarcinoma*, *Wilm's tumour*, *neuroblastoma*, *childhood rhabdomyosarcoma*, and *Ewing sarcoma*.

The variety of mechanisms by which intercalators can act make them a promising target for anti-cancer prodrugs. They can also be linked into a variety of activation strategies.

1.7 General Strategy for the Synthesis of Novel Anti-cancer Prodrugs

The main feature of a DNA intercalating agent is its chromophore, the planar section usually being composed of 3 or 4 fused coplanar rings. The planarity can be destroyed however by the incorporation of a bridge across one of the rings. We envisioned such a bridged, non-planar compound as an ideal prodrug target, with low cytotoxicity and low DNA binding affinity. Activation of such a prodrug **1.3** to a corresponding planar form **1.4**, as illustrated conceptually in Fig. 1.7.1, could result in an intercalator with both high toxicity and high DNA binding affinity.



Fig. 1.7.1

From a retrosynthetic viewpoint, Diels-Alder cycloaddition chemistry provides a convenient route to the synthesis of bridged compounds such as **1.3**.

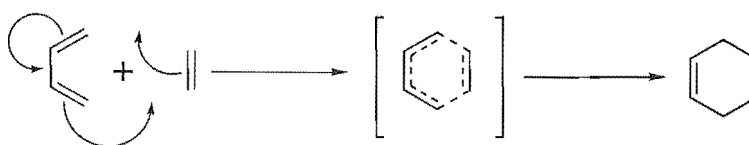


Fig. 1.7.2

The Diels-Alder reaction, as illustrated in Fig. 1.7.2, was so named after its two discoverers Otto Diels and Kurt Alder, and earned them the Nobel prize in chemistry in 1950. It is a 4+2 cycloaddition reaction between a diene and a

dienophile which generally occurs in a pericyclic fashion, proceeding through a cyclic transition state.¹⁹

The use of the Diels-Alder cycloaddition reaction to form the kind of bridged prodrug we envisioned is illustrated in Fig. 1.7.3.

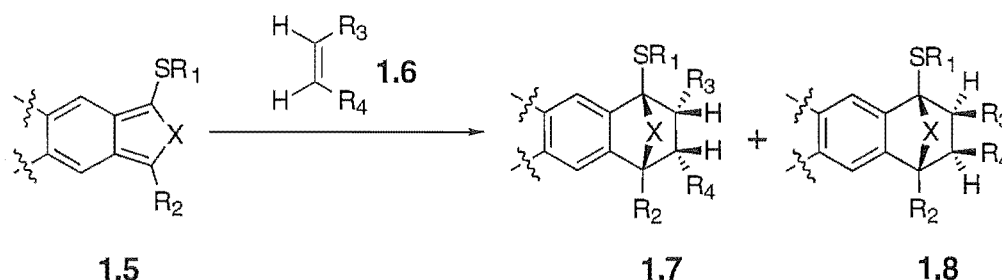


Fig. 1.7.3

Here an isobenzofuran (X=O), or an isobenzothiophene (X=S) as the diene **1.5** is reacted in Diels-Alder fashion with a dienophile **1.6**. There is the potential for two isomers, **1.7** and **1.8**, to be formed in such a cycloaddition, corresponding to the *endo* and *exo* configurations. These adducts contain an oxygen or sulfur bridge which should be relatively stable, but cleavable under particular conditions. As can be seen, various substituents can be incorporated into the reaction (R₁, R₂, R₃, and R₄), giving it great flexibility. For instance R₃ and R₄ can potentially be linked together, forming an additional ring.

Both isomers will form a compound of the type **1.9** if the bridge is opened as shown in Fig. 1.7.4. The loss of XH₂ from **1.9** results in the planar aromatic product **1.10**.

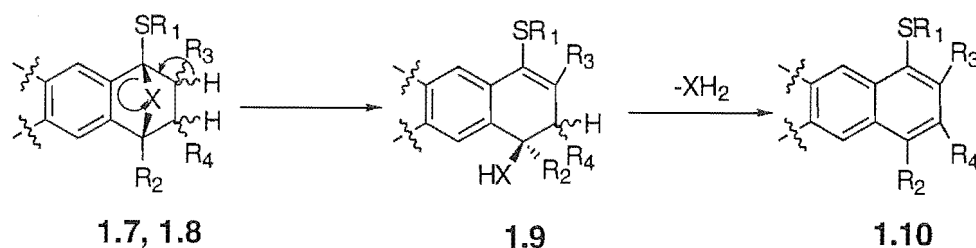


Fig. 1.7.4

In vitro, such a ring opening can be performed by using acidic catalysis. However, if the substituent R_1 is carefully chosen, then there is the potential for cleavage of the SR_1 bond to act as the trigger for aromatisation *in vivo* (cf. Section 4.9). In this way, generation of an intercalating agent can be linked to a tumour selective process, and tumour cells may be selectively targeted.

1.8 Summary

The primary synthetic goal of this thesis is the synthesis of potential non-planar prodrugs utilising Diels-Alder chemistry, along with the corresponding ring-opened planar products. Part A deals with an investigation of model Diels-Alder chemistry. Part B deals with the development of these models into potential anti-cancer compounds. It is hoped that such compounds may provide the basis for new strategies of cancer chemotherapy.

*DIELS-ALDER APPROACHES
TO ANTI-CANCER PRODRUGS*

Part A: Model Investigations

Introduction

Part A describes an investigation into the Diels-Alder chemistry of isobenzofurans with a range of dienophiles. This investigation was focused on the design of a model system for the synthesis of Diels-Alder adducts as prodrugs for anti-cancer agents. Chapter 2 describes the synthesis of a range of appropriate diene precursors. Chapter 3 examines the synthesis of a range of dienophiles, along with the synthesis of polycyclic aromatic compounds which are models for the activated prodrugs. Chapter 4 then describes an investigation into the Diels-Alder chemistry of the resulting dienes and dienophiles. A description of the bridge cleavage of the resulting adducts is also presented.

Chapter 2

DIENE PRECURSORS

2.1 Introduction

Our strategy for the synthesis of prodrugs was based around the formation of stable bridged adducts from Diels-Alder reactions. Two components are required for Diels-Alder reactions: a diene and a dienophile.

The required diene is a system containing two double bonds separated by a 'single' bond. However, because such a system is conjugated the π electron density is spread across all four atoms. In order to partake in a Diels-Alder reaction a diene must be able to adopt a *s-cis* conformation, that is a *cis*-like conformation around the central bond. This is due to the pericyclic nature of the reaction, which involves a cyclic transition state. Only in the *s-cis* conformation are the terminal carbon atoms of the diene system close enough together to react through such a cyclic transition state and form a new ring (Fig 2.1.1).



Fig. 2.1.1

The dienes under scrutiny in this work are all rigidly fixed in the correct *s-cis* geometry by their cyclic nature, being derivatives of isobenzofuran **2.1**, or isobenzothiophene **2.2**, which are shown in Fig. 2.1.2. In terms of relative aromaticity isobenzothiophenes are less aromatic in character than benzene, while isobenzofurans have even less aromatic character. Isobenzofurans are known to be highly reactive dienes in Diels-Alder cycloaddition reactions. Isobenzothiophenes are likely to be less reactive as dienes in such reactions.

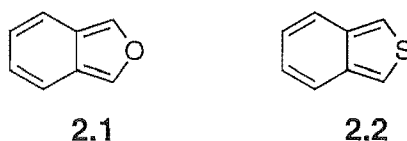


Fig. 2.1.2

The dienes were all prepared from precursors of the general structure **2.3** as shown in Fig. 2.1.3.

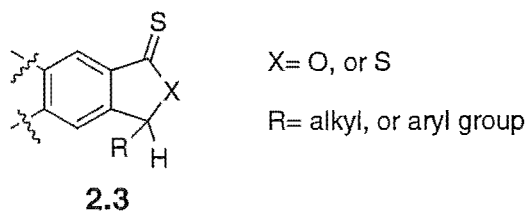


Fig. 2.1.3

These precursors were one of two main types: furanthiones (X=O) and thiophenethiones (X=S). This chapter deals with the synthesis of furanthiones and the synthesis of thiophenethiones is discussed in Chapter 7.

Three classes of furanthiones were used as diene precursors. The isobenzofuranthiones (Section 2.2), naphthofuranthiones (Section 2.3), and quinofuranthiones (Section 2.4).

2.2 Isobenzofuranthiones

The simplest of the isobenzofuranthiones synthesised was 1(3H)-isobenzofuranthione **2.5**. This was made by thiation of 1(3H)-isobenzofuranone **2.4**, also known as phthalide, by Lawesson's reagent as shown in Fig. 2.2.1.²⁰

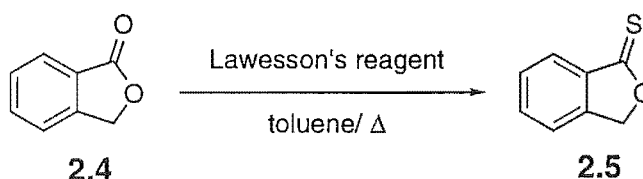


Fig. 2.2.1

Lawesson's reagent, shown in Fig. 2.2.2, is 2,4-*bis*-(*p*-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-dithione. It is commonly used to convert carbonyl groups to thiones.²¹ Another reagent which has been used successfully to perform thiations is phosphorous pentasulfide, but this has been used more commonly for exchanging the oxygen atom of a furan ring for a sulfur atom.²²

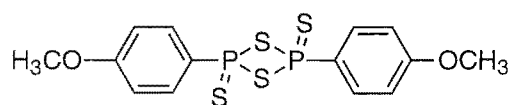


Fig. 2.2.2 Lawesson's Reagent

A phenyl-substituted derivative of **2.5**, 3-phenyl-1(3H)-isobenzofuranthione, was of particular interest as the presence of a phenyl ring at this position has been shown by Cava *et al.* to stabilise both furans and thiophenes relative to the non-substituted cases.^{23,24} Thus the isobenzofuran derived from 3-phenyl-1(3H)-isobenzofuranthione would be expected to be relatively stable.

The synthesis of 3-phenyl-1(3H)-isobenzofuranthione **2.9** is outlined in Fig. 2.2.3. Firstly isobenzofuran-1,3-dione **2.6** was ring-opened and arylated using aluminium chloride and benzene to give 2-benzoylbenzoic acid **2.7**.²⁵ 2-Benzoylbenzoic acid **2.7** was then cyclised using zinc and glacial acetic acid to give 3-phenyl-1(3H)-isobenzofuranone **2.8**.²⁶ Lawesson's reagent was again used to give 3-phenyl-1(3H)-isobenzofuranthione **2.9**.

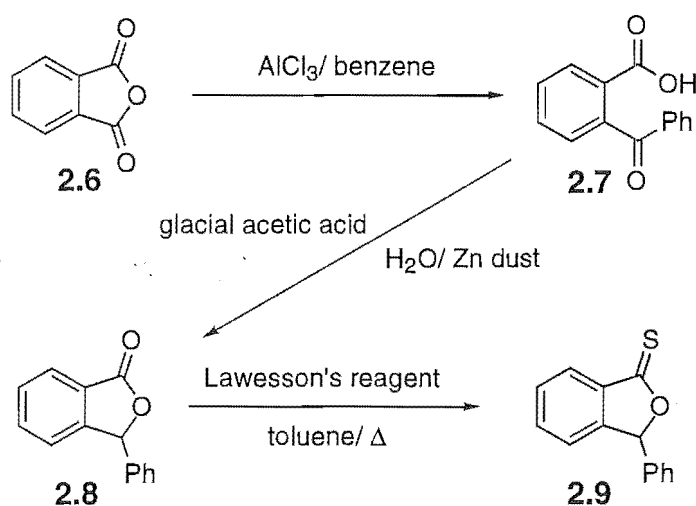


Fig. 2.2.3

A different approach was used to synthesise the 3-methyl and 3-ethyl derivatives of isobenzofuranthione. These were prepared via the intermediate 3-hydroxy-1(3H)-isobenzofuranone **2.11** as shown in Fig. 2.2.4. 1(3H)-Isobenzofuranone **2.4** was first melted and bromine was bubbled through it on a carbon dioxide carrier gas to give the brominated product 3-bromo-1-isobenzofuranone **2.10**.²⁷ Hydrolysis of **2.10** in water gave 3-hydroxy-1(3H)-isobenzofuranone **2.11**.²⁸

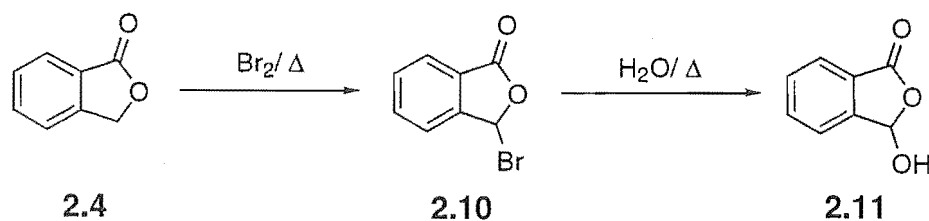


Fig. 2.2.4

3-Hydroxy-1(3H)-isobenzofuranone **2.11** exists as an equilibrium mixture of both its ring-closed **2.11a** and ring-opened **2.11b** tautomers as shown in Fig. 2.2.5. The aldehyde component reacts with a range of Grignard reagents, as demonstrated by Rodrigo.²⁹

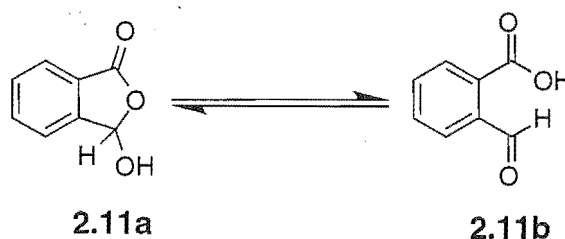


Fig. 2.2.5

Grignard reactions are nucleophilic addition reactions, but, unlike similar additions such as the nucleophilic addition of water, they are generally irreversible. Grignard reagents act as powerful nucleophiles due to the strong polarisation of the carbon-magnesium bond, with a high electron density on the carbon. Acid-base complexation of the magnesium ion with the carbonyl oxygen atom of the starting material first serves to make the carbonyl group a better electrophile as shown in Fig. 2.2.6.³⁰ Nucleophilic addition to **2.11b** then produces a tetrahedral intermediate **2.12**. Protonation of the intermediate by aqueous acid gives the neutral alcohol **2.13**.

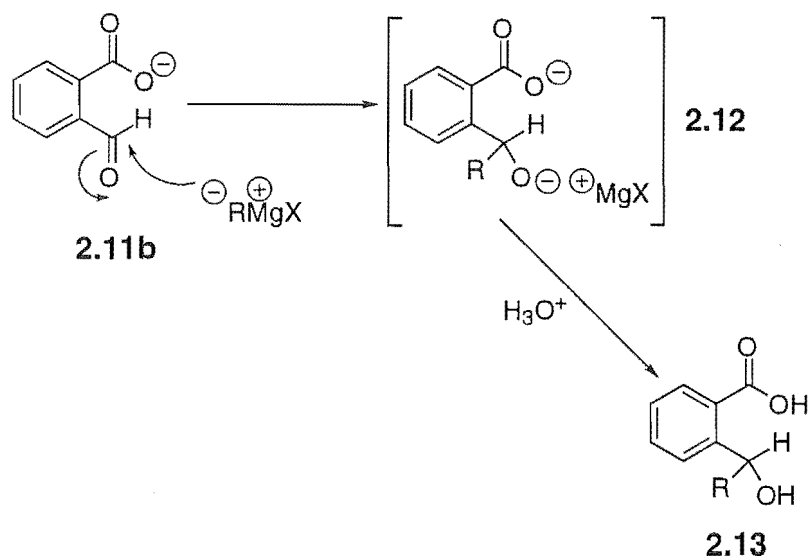


Fig. 2.2.6 Proposed mechanism of the Grignard reaction

The alcohols formed in this manner from **2.11**, with either methyl or ethyl substitution at the 3-position, were then dehydrated to give the corresponding ring-closed products **2.14a** and **2.14b** as shown in Fig. 2.2.7. Both these products were yellow oils which were purified by radial silica chromatography.

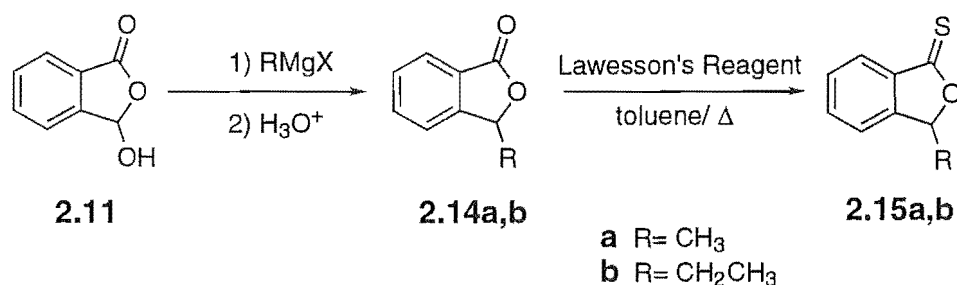


Fig. 2.2.7

Reaction of **2.14a** and **2.14b** with Lawesson's reagent was found to give the corresponding isobenzofuranthiones **2.15a** and **2.15b**, which were also yellow oils. These were now available for use as diene precursors.

2.3 Naptho[2,3-c]furan-1-thiones

Naphthalene-2,3-dicarboxylic anhydride was used as the starting material for the synthesis of naphthofuranthiones. The synthesis of naphthalene-2,3-dicarboxylic anhydride itself was carried out in 3 steps as outlined in Fig. 2.3.1.

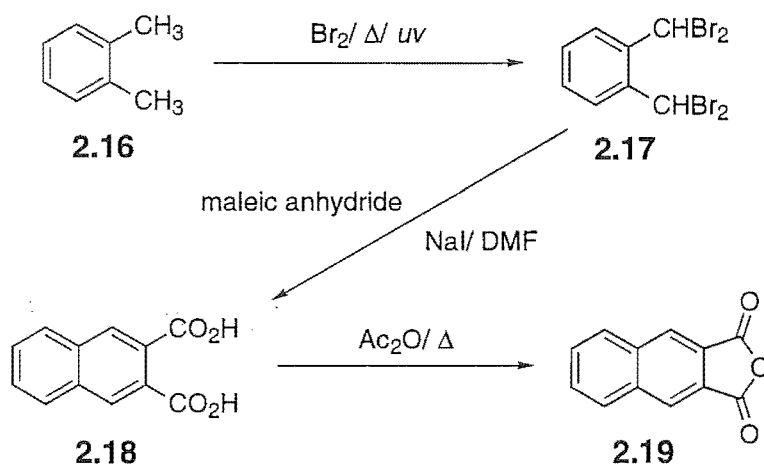


Fig. 2.3.1

Firstly, a standard bromination of 2-xylene **2.16** under ultraviolet light led to the formation of 1,2-*bis*(dibromomethyl)benzene **2.17**.³¹ 1,2-*bis*-(Dibromomethyl)-benzene **2.17** has been shown to be capable of undergoing a concerted elimination reaction, forming the highly reactive 2-quinodimethane derivative **2.20** when reacted with sodium iodide as shown in Fig. 2.3.2.³²

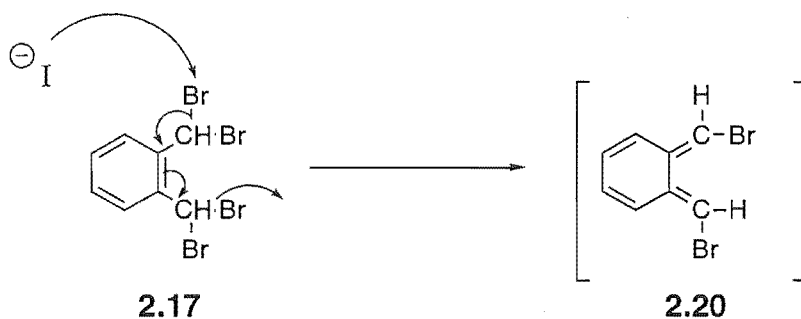


Fig. 2.3.2

This feature was exploited and the intermediate **2.20**, an excellent diene, was immediately trapped with the dienophile maleic anhydride in a Diels-Alder reaction to give naphthalene-2,3-dicarboxylic acid **2.18** as the Diels-Alder adduct.³³ Conversion of **2.18** to naphthalene-2,3-dicarboxylic anhydride **2.19**, by refluxing with acetic anhydride proved facile and this step proceeded in quantitative yield.

To make the diene precursor 1,3-dihydronaphtho[2,3-*c*]furan-1-thione **2.22**, naphthalene-2,3-dicarboxylic anhydride **2.19** was first reduced to 1,3-dihydronaphtho[2,3-*c*]furan-1-one **2.21** as shown in Fig. 2.3.1.

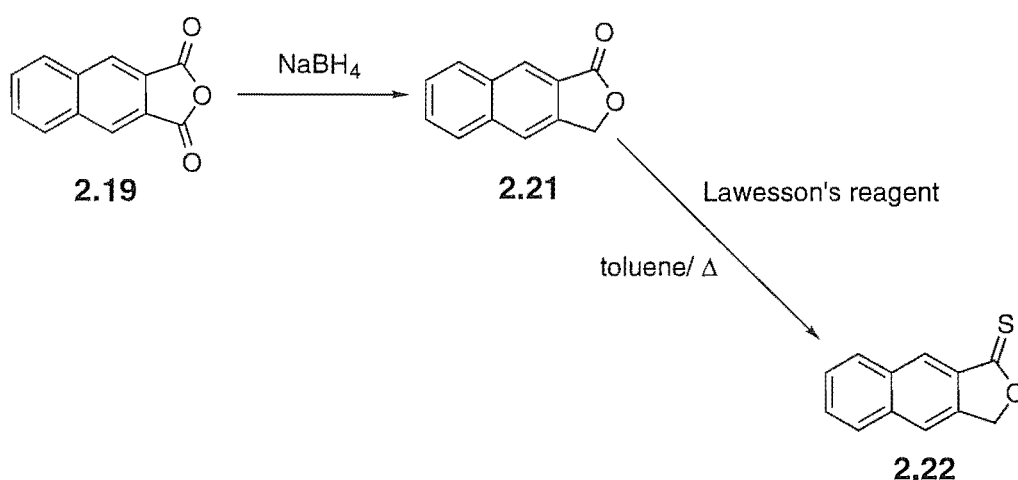


Fig. 2.3.1

This reduction was performed using sodium borohydride, a commonly used reagent for reduction of cyclic anhydrides to lactones.³⁴ Thiation of **2.21** with Lawesson's reagent gave 1,3-dihydronaphtho[2,3-c]furan-1-thione **2.22**.

To introduce a phenyl group at the 3-position of the naphthofuranthione, and hence potentially add stability to the subsequent diene, a method analogous to the synthesis of 3-phenyl-1(3H)-isobenzofuranthione **2.9** was employed (cf. Fig. 2.2.3).

As shown in Fig. 2.3.2, firstly naphthalene-2,3-dicarboxylic anhydride **2.19** was ring-opened and arylated using aluminium chloride and benzene to give the acid **2.23**; this was then reduced and cyclised *in situ* using zinc and glacial acetic acid to give 3-phenyl-1(3H)-isonaphtho[2,3-c]furan-1-one **2.24**.^{25,26} Reaction of **2.24** with Lawesson's reagent gave the desired product 3-phenyl-1(3H)-isonaphtho[2,3-c]furan-1-thione **2.25**.

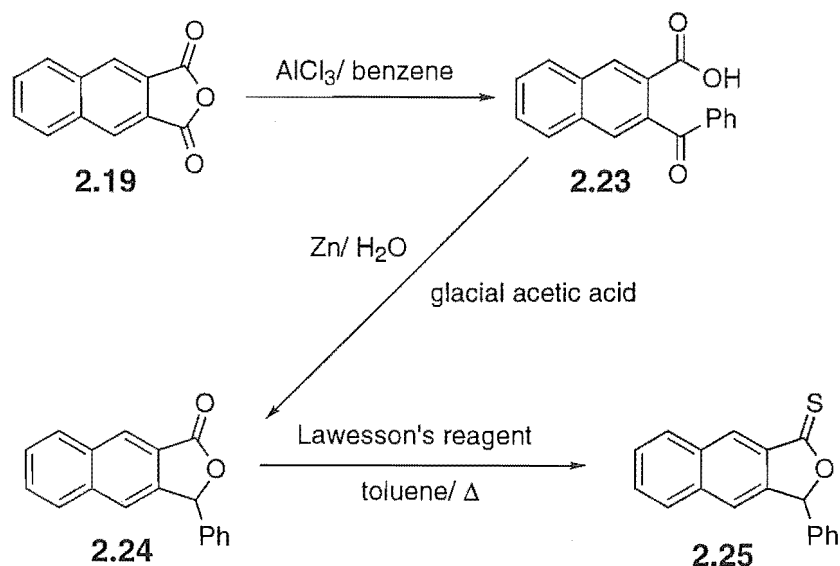


Fig. 2.3.2

By this chemistry, 1,3-dihydronaphtho[2,3-c]furan-1-thione **2.22** and 3-phenyl-1(3H)-isonaphtho[2,3-c]furan-1-thione **2.25** were available for use as diene precursors.

2.4 Quino[2,3-c]furan-1-thione

It has been noted that the presence of one or more nitrogen atoms in the chromophore of an intercalating agent often increases its binding affinity to DNA. Many intercalators of this nature are known, for example 6H-pyrido[4,3-b]carbazole **2.26** and the ellipticine derivative **2.27** which are shown in Fig. 2.4.1.⁸

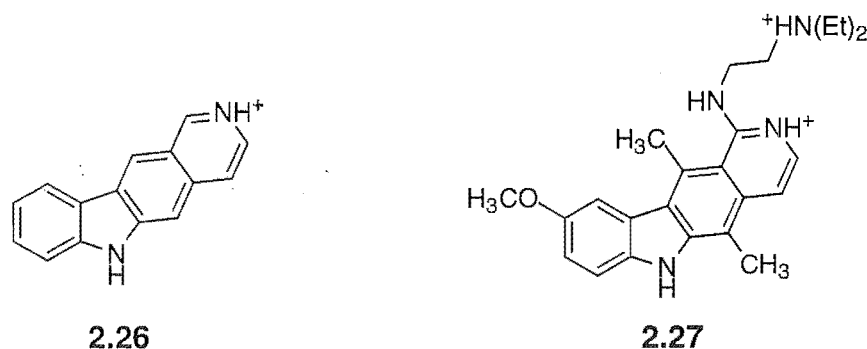


Fig. 2.4.1

The increased DNA affinity of such heterocyclic intercalators is attributed to favourable electrostatic and hydrogen bonding interactions between the protonated nitrogen atom of the chromophore and the DNA.

Quino[2,3-c]furan-1-thione, with a nitrogen atom substituted into the coplanar ring system of the furanthione, proved to be the most challenging of the furanthiones to synthesise. In order to introduce the nitrogen atom into the ring system, 2-nitrobenzaldehyde **2.28** was used as the starting material. Reduction of the nitro group using ferrous sulfate and ammonia solution gave 2-aminobenzaldehyde **2.29** which was separated out of the reaction mixture by steam distillation.³⁵ This reaction sequence is outlined in Fig. 2.4.2.

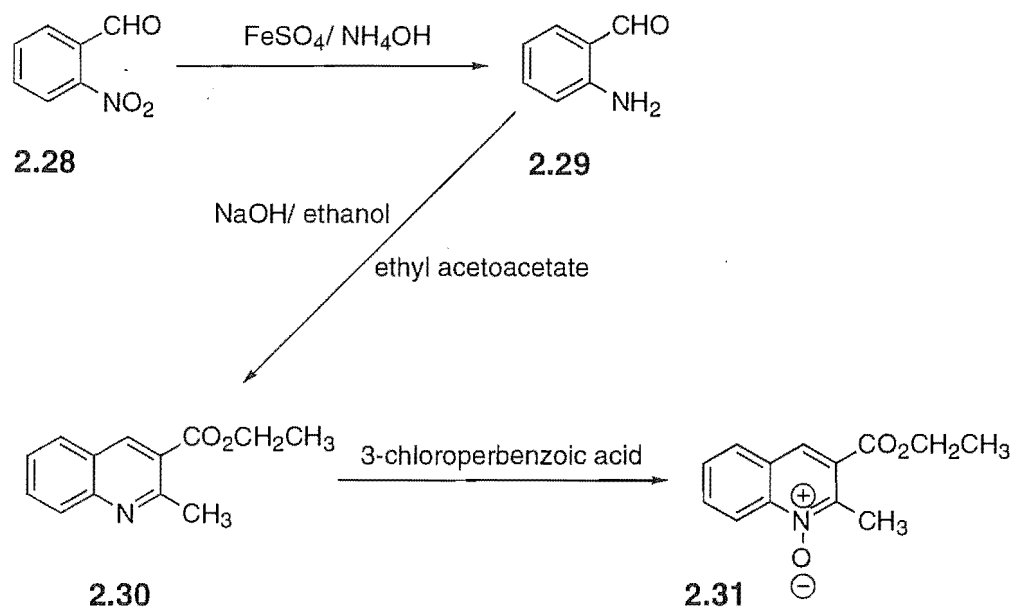


Fig. 2.4.2

A variation of the Friedländer synthesis was then employed to cyclise 2-aminobenzaldehyde **2.29** with ethyl acetoacetate to give **2.30**.³⁶

The Friedländer synthesis is an acid or base catalysed reaction between a 2-amino substituted aromatic aldehyde or ketone with an appropriately substituted aldehyde, ketone, or other carbonyl containing compound with a reactive α -methylene group. It involves condensation followed by cyclodehydration. Quinoline formation was carried out effectively by stirring the reactants in ethanol at room temperature with a catalytic amount of sodium hydroxide present.^{37,38,39,40}

The next step necessary was the conversion of **2.30** to the corresponding *N*-oxide **2.31** using 3-chloroperbenzoic acid.

The first quinoline *N*-oxide was prepared by Meisenheimer in 1926 by oxidation of quinoline with perbenzoic acid.⁴¹ Ochiai *et al.* later established the necessity of an acid in this kind of oxidation as well as the conditions which have been most commonly used since.⁴² Usually a mixture of glacial acetic with 30% hydrogen peroxide is satisfactory, however for lower temperature reactions 3-

chloroperbenzoic acid has been used effectively, and this is the reagent we chose.^{43,44,45} A possible mechanism of *N*-oxide formation by oxidation of a quinoline is shown in Fig. 2.4.3.

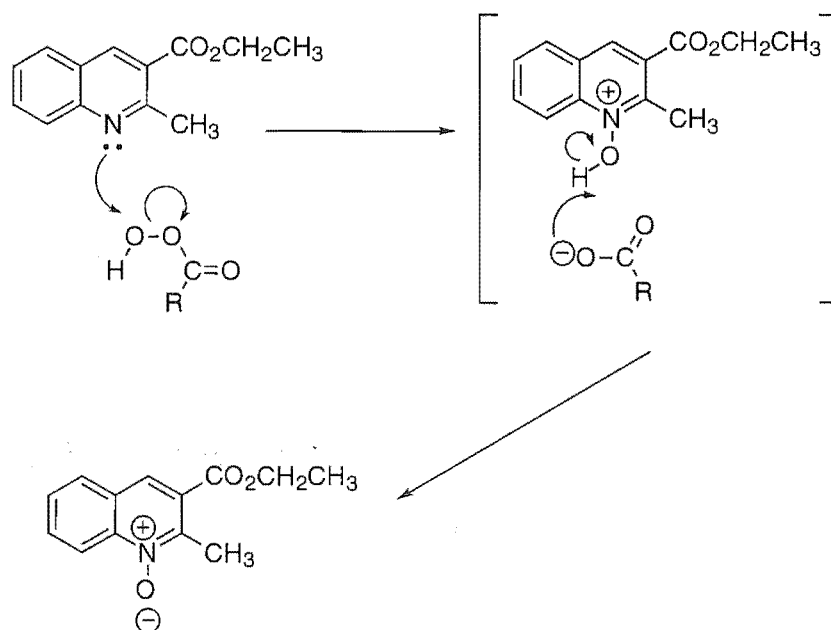


Fig. 2.4.3 Possible mechanism of *N*-oxide formation

The reaction of an alkyl-substituted quinoline *N*-oxide with acetic anhydride has been shown to result in deoxygenation of the quinoline nitrogen and acetoxylation of the alkyl group.⁴⁶ This characteristic reaction of *N*-oxides was used to synthesise **2.32** from **2.31**, as shown in Fig. 2.4.4.

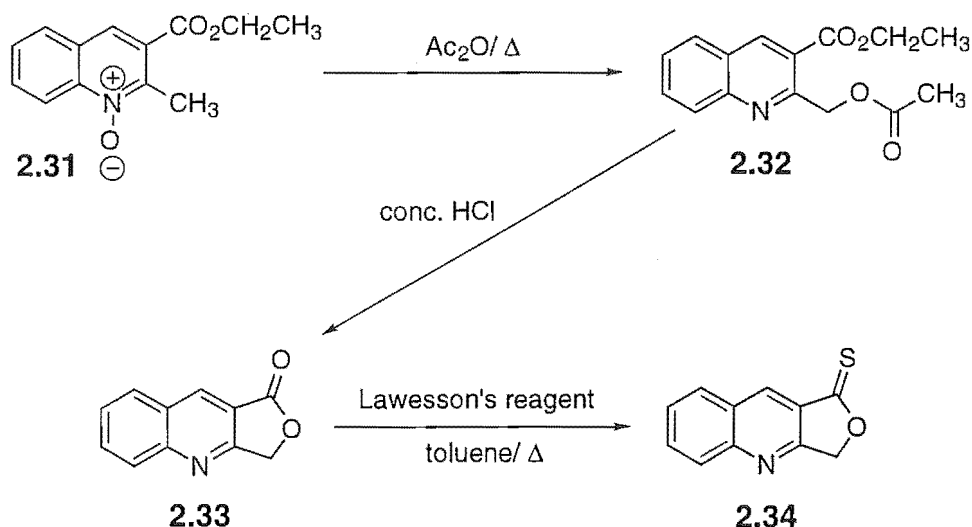


Fig. 2.4.4

Cyclisation of **2.32** to the lactone quino[2,3-c]furan-1-one **2.33** was performed with concentrated hydrochloric acid.⁴⁷ Thiation of **2.33** using Lawesson's reagent gave the desired diene precursor quino[2,3-c]furan-1-thione **2.34**, although in lower yield than that obtained for 1,3-dihydronaphtho[2,3-c]furan-1-thione **2.22**. This was now available for use as a diene precursor.

2.5 Summary

Using known chemistry a range of furanthione diene precursors, both bicyclic and tricyclic, was synthesised. These were: isobenzofuranthione, along with its 3-phenyl, 3-methyl, and 3-ethyl substituted derivatives; naphthofuranthione, along with its 3-phenyl substituted derivative; and a heterocyclic quino[2,3-c]furanthione. The conversion of these diene precursors to dienes and their subsequent trapping with dienophiles to form Diels-Alder adducts will be described in Chapter 4.

Chapter 3

DIENOPHILES

3.1 Introduction

The alkene component of a Diels-Alder cycloaddition reaction is called a dienophile, meaning 'diene lover'. The cycloaddition is most efficient when the dienophile has complementary electronic properties to the diene. For a normal electron-demand Diels-Alder reaction, which has an electron-rich diene, this means that the reaction occurs optimally if the dienophile is substituted by an electron withdrawing group.¹⁹ Hence the reaction is biased towards those dienophiles where the double bond is next to a substituent which withdraws electron density. For an inverse electron-demand Diels-Alder reaction the situation is reversed.

Many different classes of compounds have been used as dienophiles in Diels-Alder chemistry. For our purposes, the choice of dienophile was crucial, as it would determine to a large extent the effectiveness of our final planar aromatic compounds as intercalators. We were interested in incorporating a range of functional groups into these aromatic compounds via the dienophile, in particular nitrogen based substituents.

This chapter describes the dienophiles used in Part 1 of this thesis and, where appropriate, their synthesis. Section 3.2 describes those simple dienophiles used initially, which were related to dimethyl fumarate and dimethyl acetylenedicarboxylate. Section 3.3 describes the synthesis of *N*-aryl maleimides. Section 3.4 describes the extension of the *N*-aryl maleimide synthesis to polycyclic compounds related to the products of the proposed Diels-Alder chemistry.

3.2 Fumarate and Acetylene Based Dienophiles

Dimethyl fumarate **3.1** and dimethyl acetylenedicarboxylate **3.2** are commonly used dienophiles and are readily available reagents (Fig. 3.2.1).

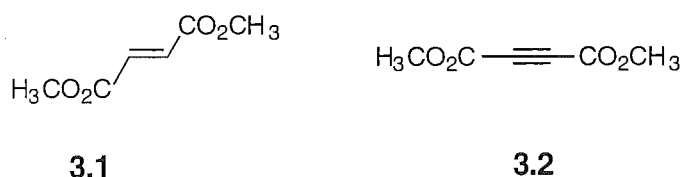


Fig. 3.2.1

Amide analogues of fumaric acid were generated as illustrated in the reaction scheme shown in Fig. 3.2.2. Fumaric acid **3.3** was first chlorinated using phosphorous pentachloride to give the highly reactive fumaroyl dichloride **3.4**.⁴⁸ Reaction of **3.4** with *n*-butylamine and *N,N*-dimethylethylene diamine gave the products **3.5** and **3.6** respectively.⁴⁹

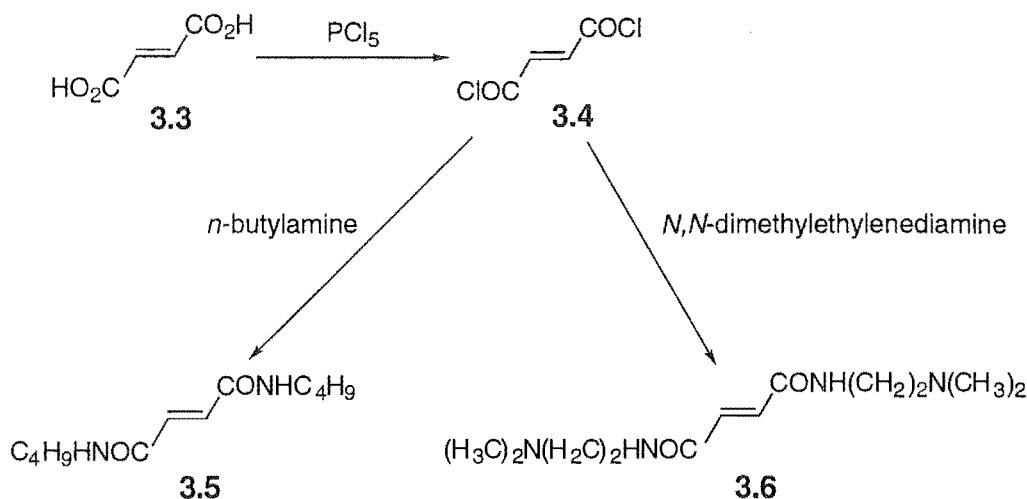


Fig. 3.2.2

The two diamides 3.5 and 3.6, along with dimethyl fumarate and dimethyl acetylenedicarboxylate, were used as dienophiles in Diels-Alder reactions which are described in Chapter 4.

3.3 *N*-Aryl maleimides

N-Phenyl maleimide is well-known as an excellent dienophile. In addition, it offers the possibility of incorporating useful functionality into the Diels-Alder adducts via substitution on the phenyl ring.

N-Phenyl maleimide is synthesised in a two stage process from maleic anhydride and aniline as shown in Fig 3.3.1, which also serves to illustrate the synthesis of *N*-aryl maleimides in general.⁵⁰

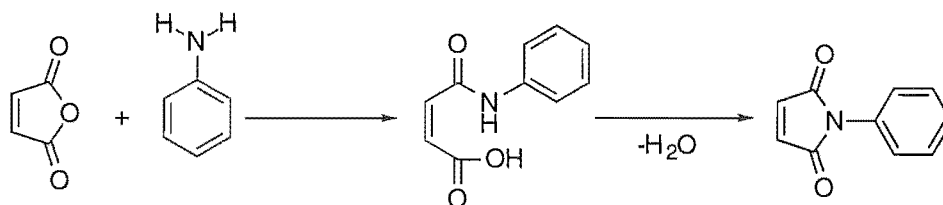


Fig. 3.3.1 Two stage synthesis of *N*-aryl maleimides

In the first step nucleophilic attack on the maleic anhydride by the nitrogen of aniline results in the formation of an unsymmetrical intermediate. Dehydration of this intermediate by heating gently with acetic anhydride results in the symmetrical product *N*-phenyl maleimide. *N*-Phenyl maleimide is easily identified with proton NMR by its characteristic singlet at 6.85ppm, corresponding to its two olefinic protons. In fact, the presence of a singlet in this region is characteristic of all *N*-aryl maleimides.

An analogous procedure was followed to produce nitro-substituted *N*-aryl maleimides, with 2-nitroaniline and 3-nitroaniline respectively used as starting materials. This led to the synthesis of **3.7** and **3.8** as shown in Fig. 3.3.2.

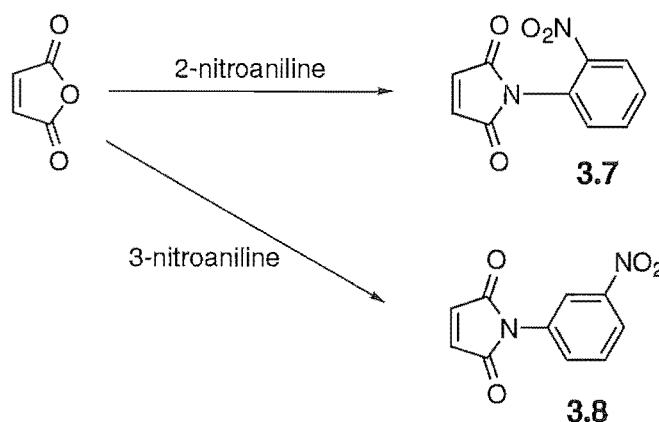


Fig. 3.3.2

It was envisioned that appropriate substitution of *N*-aryl maleimides could lead to Diels-Alder adducts with stronger binding affinity to DNA. One possible route to such compounds was via amide bond formation from amine substituted *N*-aryl maleimide starting materials.

An attempt was therefore made to reduce the nitro-substituted derivative **3.8** to the corresponding amino-substituted compound **3.9** as shown in Fig. 3.3.3.

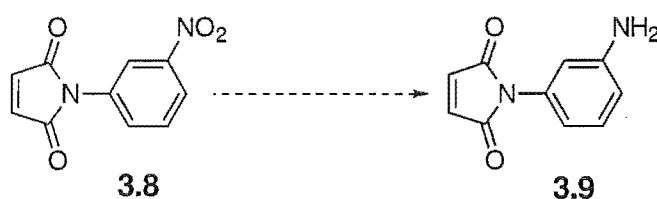


Fig. 3.3.3.

Common reagents which have been used for the reduction of a nitro group include tin and hydrochloric acid, iron and acetic acid, and catalytic agents.^{51,52,53,54,55} In view of possible side-reactions we chose to evaluate tin and hydrochloric acid. Several attempts to perform this reduction however, using both the *meta*-substituted **3.8**, and the *ortho*-substituted **3.7**, were unsuccessful, with the maleimide ring being opened in the reaction.

A different approach to synthesise amide substituted *N*-aryl maleimides was therefore used. In this approach, outlined in Fig. 3.3.4, the maleimide ring was incorporated in the final step, after any necessary reductions.

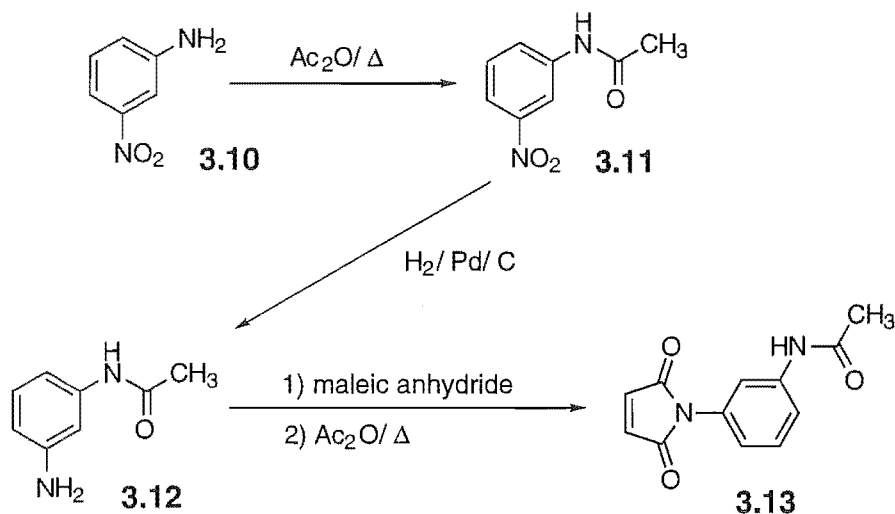


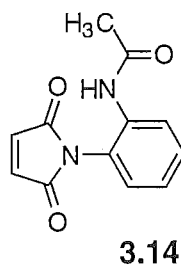
Fig. 3.3.4

3-Nitroaniline **3.10** was first refluxed with acetic anhydride to give the acetylated product **3.11**. Potentially, any acyl substituent could be added in this step, allowing a wide range of functional groups to be incorporated into subsequent Diels-Alder adducts.

The next step again required reduction of the nitro group. The procedure using tin and hydrochloric acid proved unsuitable once more. So catalytic hydrogenation was chosen. Nitro groups have been shown to be one of the most easily reduced groups by catalytic hydrogenation.⁵⁶ Catalysts which have been successfully employed for the reduction of aromatic nitro groups include platinum oxide, Raney nickel, and palladium on carbon.⁵²

We performed the reduction of **3.11** to **3.12** by dissolving **3.11** in methanol, and using 5% palladium-on-carbon as the catalyst for hydrogenation. The amine **3.12** thus formed was then reacted with maleic anhydride followed by acetic anhydride, to give the desired product **3.13**.⁵⁰

The corresponding *ortho*-substituted compound **3.14** was synthesised in an analogous manner, starting with 2-nitroaniline (Fig. 3.3.5).

**Fig. 3.3.5**

The two compounds thus synthesised, *N*-(3-acetylaminophenyl)maleimide **3.13** and *N*-(2-acetylaminophenyl)maleimide **3.14** were now available for use as dienophiles in Diels-Alder chemistry. This chemistry is described in Chapter 4.

3.4 Planar Aromatic Models

3.4.1 Introduction

Section 3.3 described the successful syntheses of *N*-aryl maleimides. Diels-Alder adducts resulting from the use of *N*-substituted maleimides as the dienophiles will produce polycyclic aromatic derivatives containing a cyclic imide structure. The chemistry used to generate maleimides can also be used to generate analogous polycyclic structures directly. This section describes the syntheses of planar aromatic models, as a preliminary survey to see whether any class of *N*-substituted imide might offer interesting biological opportunities above those offered by simple *N*-aryl imides. A range of *N*-substituted imides was therefore prepared.

The synthesis of *N*-substituted imides can be carried out in several ways, the two most favoured being a two-step synthesis, as in the preparation of *N*-phenyl maleimide, with nucleophilic attack in the first step being followed by cyclisation in the second, or a single step synthesis in which both processes take place in the

same reaction vessel, such as in the reported synthesis of 3-isopropyl-*N*-(benzyloxy)succinimide.^{50,57} The latter route was chosen for our purposes and the reaction conditions tested by the insertion of *N,N*-dimethylethylene diamine into isobenzofuran-1,3-dione **2.6** as shown in Fig. 3.4.1.

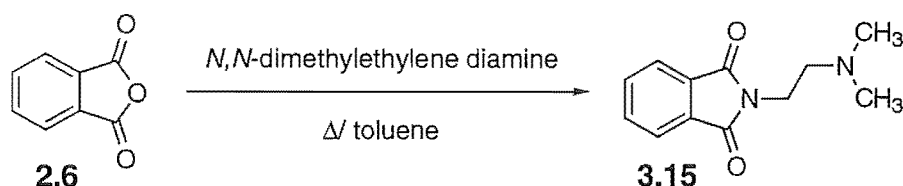


Fig. 3.4.1

In this method the anhydride, isobenzofuran-1,3-dione **2.6** in this case, is brought to reflux in toluene and the amine added and further refluxed. This reaction proceeded cleanly to give **3.15** in 90% yield.

Our work was directed at the synthesis of polycyclic systems, so quinoline-2,3-dicarboxylic anhydride and naphthalene-2,3-dicarboxylic anhydride were chosen as the starting anhydrides. Section 3.4.2 describes the attempted synthesis of quinoline-2,3-dicarboxylic anhydride. Section 3.4.3 describes the synthesis of polycyclic *N*-substituted imide derivatives from naphthalene-2,3-dicarboxylic anhydride.

3.4.2 Quinoline-2,3-dicarboxylic anhydride

As noted in Section 2.4 the presence of a nitrogen atom in the chromophore of an intercalating agent often increases its binding affinity to DNA. Quinoline-2,3-dicarboxylic anhydride was therefore targeted as a useful starting material for the synthesis of *N*-substituted imides. Our attempted method of synthesising quinoline-2,3-dicarboxylic anhydride is outlined in Fig. 3.4.2.

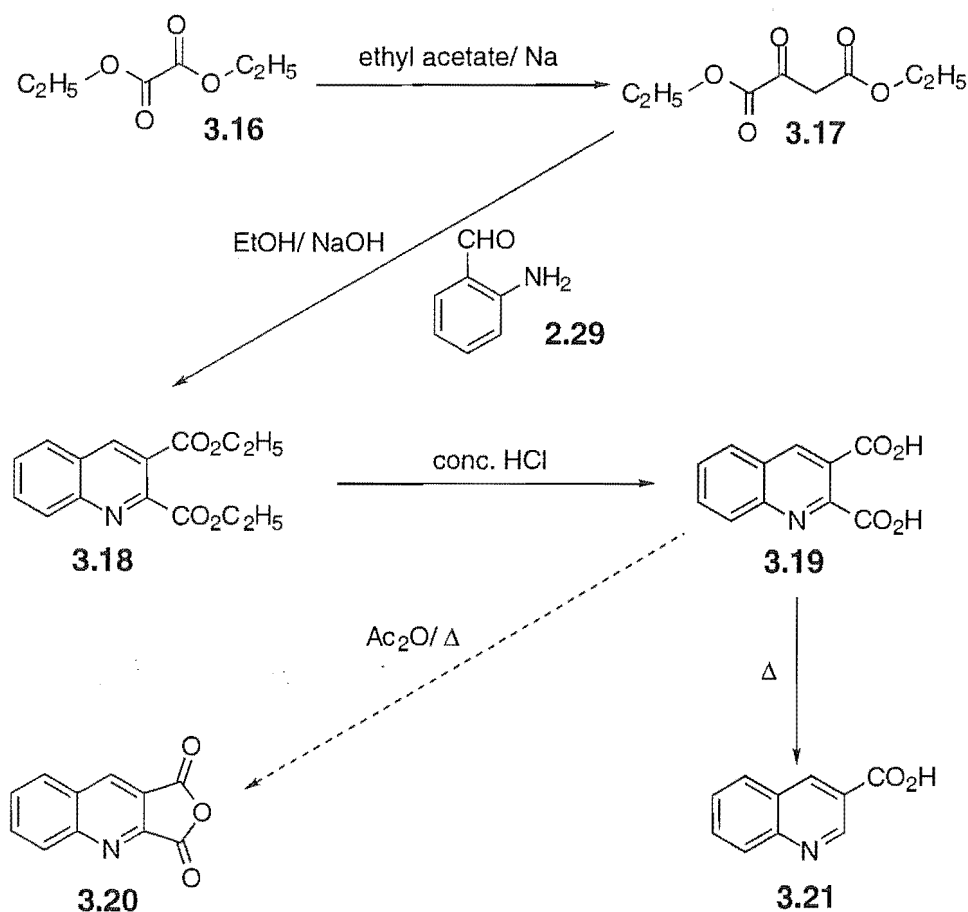


Fig. 3.4.2

In the first step diethyl oxalate **3.16** was reacted with ethyl acetate and sodium metal to give the sodium salt of diethyl oxaloacetate **3.17**. This was recrystallised and converted to its neutral form by addition of hydrochloric acid and extraction with ether. The Friedländer synthesis was then used to react **3.17** with 2-aminobenzaldehyde **2.29**, prepared freshly from 2-nitrobenzaldehyde **2.28** (cf. Fig. 2.4.2), forming the diethyl derivative of 2,3-quinoline dicarboxylic acid **3.18**.^{36,58} The conditions of the Friedländer synthesis were the same as those used successfully in Section 2.4, namely stirring the reactants in ethanol at room temperature with a catalytic amount of sodium hydroxide present.

With **3.18** thus synthesised, quinoline-2,3-dicarboxylic acid **3.19** itself was prepared by removal of the ethyl substituents with concentrated hydrochloric

acid. However the proposed final step of synthesising quinoline-2,3-dicarboxylic anhydride **3.20** from the diacid **3.19** was unsuccessful. Reflux of **3.19** with acetic anhydride was found to result in thermal decomposition, with decarboxylation resulting in formation of 3-quinoline carboxylic acid **3.21**.^{59,60}

3.4.3 Derivatives of Naphthalene-2,3-dicarboxylic anhydride

The synthesis of naphthalene-2,3-dicarboxylic anhydride **2.19** is described in Section 2.3. The preparation of a range of polycyclic *N*-substituted imides from **2.19** is summarised in Fig. 3.4.3.

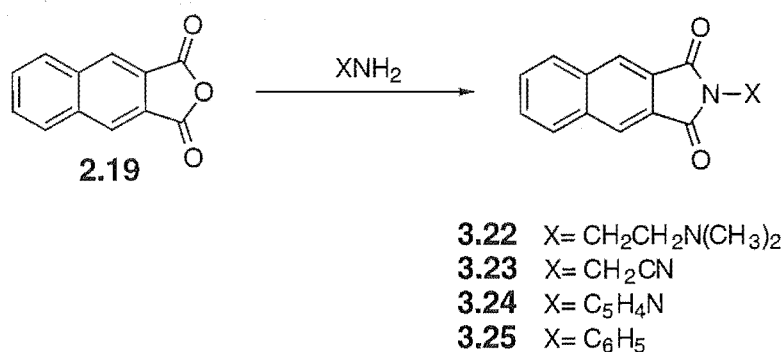


Fig. 3.4.3

The amines were selected so as to incorporate a variety of functionalities and thereby explore the effects of various substituents on the properties of the polycyclic imides relative to the *N*-phenyl case **3.25**. Studies of intercalation have shown that the cytotoxicity of an intercalating agent often depends not only on the presence of a coplanar aromatic ring system, but also on the presence of a substituent capable of interacting with and affecting DNA biochemistry.⁸ Thus **3.22**, **3.23**, and **3.24** all incorporate a heteroatom, nitrogen, separate from the chromophore itself, with the aim of enhancing favourable interactions between the DNA and the intercalator. For example the dimethylamine moiety of **3.22**,

which can be protonated, has been shown able to produce favourable electrostatic interactions with phosphate groups on the DNA backbone, if positioned correctly in relation to the DNA.⁶¹ Similarly cyano and pyridinyl groups, such as those incorporated into **3.23** and **3.24** respectively, are capable of hydrogen bonding interactions with the backbone of DNA.

The polycyclic *N*-substituted imides thus synthesised were tested for biological activity in the P388 anti-tumour assay (cf. Appendix). The *N*-phenyl substituted imide **3.25** showed mild anti-cancer activity, giving an ID₅₀ value of 4.39 µg/mL. However, none of the other compounds synthesised were found to have enhanced biological activity relative to the *N*-phenyl case. They were, therefore, not pursued further.

3.5 Summary

The initial dienophiles used in this thesis were dimethyl acetylenedicarboxylate, dimethyl fumarate, and amide derivatives of fumaric acid. A convenient route was developed for the synthesis of a range of *N*-aryl maleimide dienophiles, with the potential for introducing further functionality (cf. Chapter 5). A range of polycyclic *N*-substituted imides was synthesised as preliminary models of bridge-cleaved Diels-Alder adducts. These did not appear to offer any biological opportunities above those offered by *N*-aryl imides.

The Diels-Alder chemistry of the dienophiles synthesised in this chapter will be described in Chapter 4.

Chapter 4

OXYGEN-BRIDGED DIELS- ALDER ADDUCTS

4.1 Isobenzofurans

In 1964 the brief existence of isobenzofuran **4.1** was conclusively demonstrated (Fig. 4.1.1).⁶² Since then many isobenzofurans have been synthesised. Most isobenzofurans, like **4.1**, are too unstable to be isolated in the conventional sense.²⁹ Aryl substituents on the furanoid ring have a stabilising effect on the system, as do electron withdrawing groups; thus 1,3-diphenyl isobenzofuran **4.2** is a commercially available material.⁶³ Isobenzofurans with a single aryl substituent however, are not stable enough for routine isolation. It is for this reason that isobenzofurans are generally prepared and reacted *in situ*.

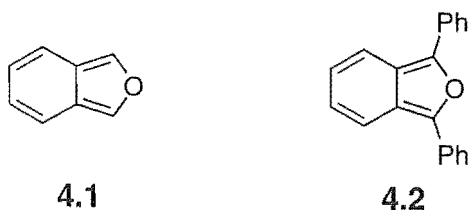


Fig. 4.1.1

Isobenzofurans are highly reactive dienes in Diels-Alder cycloadditions. Routes that have been used to synthesise isobenzofurans for subsequent trapping with dienophiles vary and include flash vacuum pyrolysis of dihydro-substituted furan precursors, the acid catalysed reaction of acetals, thermal cycloreversion, and lithium diisopropylamide (LDA) induced 1,4-elimination of acetals.^{64,65,66}

In order to synthesise sulfur substituted isobenzofurans we chose a synthetic route analogous to that Iwao *et al.* reported in pioneering the synthesis of silyloxyisobenzofurans from isobenzofuranones (Fig. 4.1.2).^{67,68} They described deprotonation of isobenzofuranones **4.3** by LDA to generate 3-lithioisobenzofuranones, silylation of the resulting ambident anions led to 1-silyloxyisobenzofurans **4.4**, due to silicon's much stronger affinity for oxygen than for carbon.⁶⁹ The silyloxyisobenzofurans so generated were trapped with simple dienophiles, such as dimethyl fumarate, to give bridged Diels-Alder adducts.

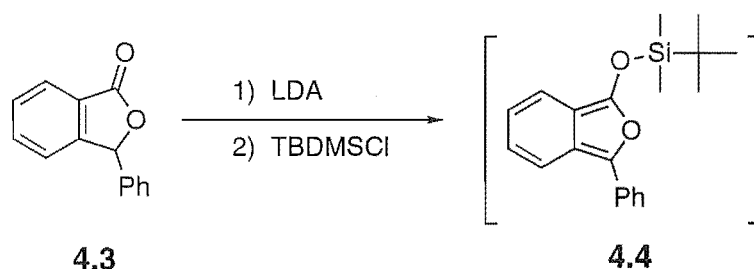


Fig. 4.1.2

Bailey *et al.* have shown that such an approach can be successfully adapted to the synthesis of sulfur-substituted isobenzofurans.⁶⁷ This strategy involved preparing anions by the deprotonation of isobenzofuranthiones with LDA, followed by alkylation on the sulfur atom. Section 4.2 will describe the general method which was followed in this thesis to generate such sulfur-substituted isobenzofurans *in situ*.

4.2 General Method for the Preparation of Isobenzofuran Dienes

The first step in the conversion of diene precursors to their corresponding dienes is deprotonation using lithium diisopropylamide (LDA, **4.6**, Fig. 4.2.1). LDA is an exceedingly powerful base, since diisopropylamine has a pK_a of 40. It is soluble in organic solvents such as tetrahydrofuran (THF), yet it is too bulky to act as an effective nucleophile.

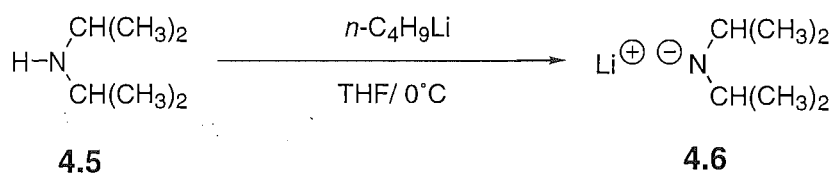


Fig. 4.2.1 Preparation of LDA.

As shown in Fig. 4.2.2, deprotonation of a isobenzofuranone **4.7** with LDA leads to formation of the diene system **4.8** as an anion. Alkylation on sulfur is then achieved using an appropriate alkylating agent, such as methyl iodide, to give the sulfur-substituted isobenzofuran **4.9**, ready for use in a Diels-Alder cycloaddition reaction. This reaction sequence is sensitive to water and must be done in an anhydrous environment.

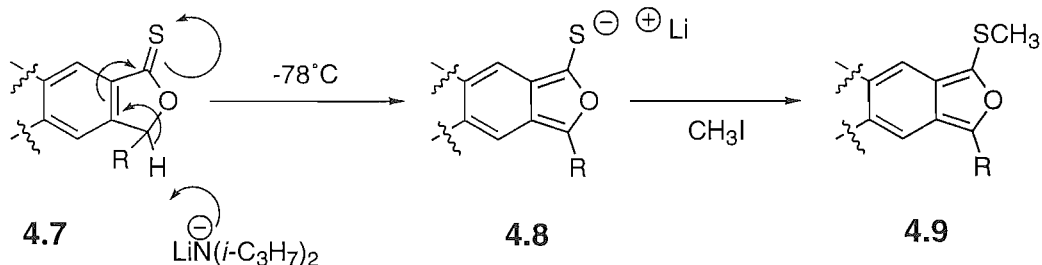


Fig. 4.2.2

The sulfur-substituted isobenzofurans thus formed were reacted immediately with a dienophile *in situ*, and no attempt was made to isolate them. The advantage of this approach was a 'one-pot' synthesis in which all the reactions involved in converting the diene precursors to Diels-Alder adducts were performed together.

4.3 Overview

This section provides an overview of the chemistry presented in this chapter. The reactions described can be grouped into two categories: the formation of Diels-Alder adducts, and the bridge cleavage of these adducts and their subsequent chemistry.

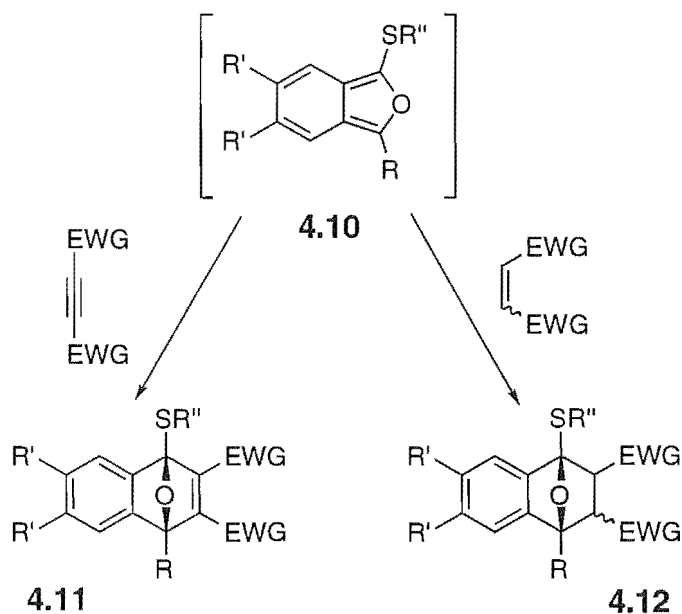


Fig. 4.3.1 Adduct formation

Fig. 4.3.1 outlines Diels-Alder adduct formation, with the diene **4.10** reacting with an alkyne or alkene dienophile to give the Diels-Alder adducts **4.11** and **4.12** respectively. The four major variable substituents present in these reaction sequences are R , R' , R'' , and EWG.

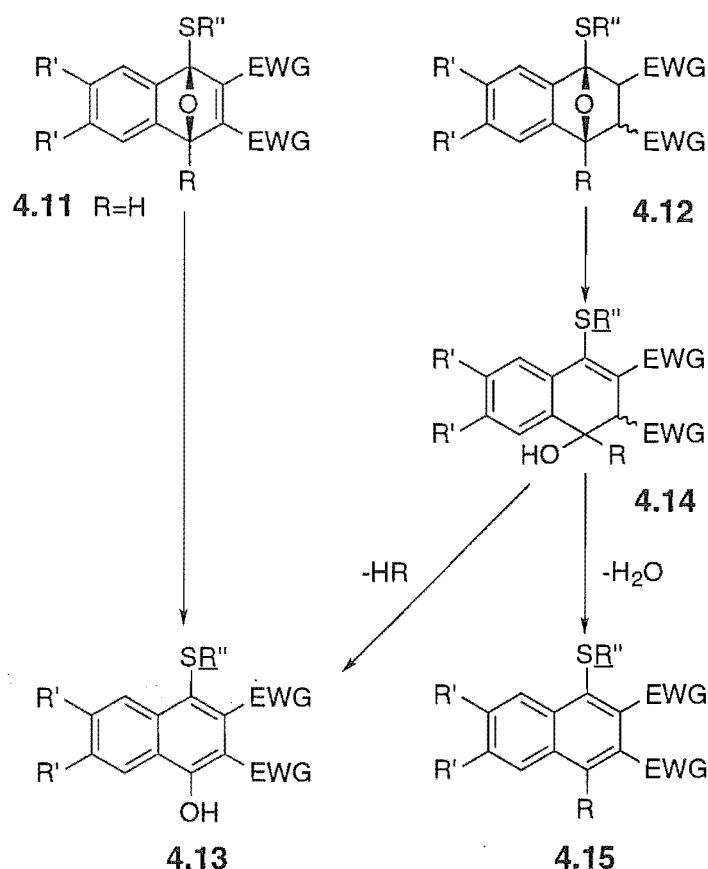


Fig. 4.3.2 Bridge cleavage and subsequent chemistry

Fig. 4.3.2 outlines some possible bridge cleavages and subsequent chemistry of these Diels-Alder adducts. Bridge cleavage can be induced by either acid catalysis, or cleavage of SR'' (in which case $R''=H$). If $R=H$, bridge cleavage of **4.11** leads to the aromatic product **4.13**. Bridge cleavage of **4.12** leads to the intermediate product **4.14**, which can follow either of two pathways to aromatisation: loss of H_2O to give **4.15**, or loss of HR to give **4.13** in the case where R is a leaving group.

This chapter describes an investigation into the effects of varying the substituents R , R' , R'' , and EWG. Sections 4.4 to 4.6 describe the effects of varying R , which has an influence on both the stability of the diene **4.10** and the pathways open to aromatisation. Initially, Sections 4.4 and 4.5 describe the Diels-

Alder chemistry of a range of simple 1-methylsulfanyl-isobenzofurans with simple dienophiles, in particular dimethyl fumarate, dimethyl acetylenedicarboxylate, and *N*-phenyl maleimide. Section 4.6 then describes an example of Diels-Alder chemistry when R is a leaving group.

Section 4.7 is focused on studies in which R' is varied, extending the ring system. R' influences both the stability of the diene **4.10**, and the biological properties of the final aromatic product.

Section 4.8 describes investigations into the effects of varying the electron withdrawing groups (EWG) of the dienophile. The choice of EWG influences the reactivity of the dienophile, and is also a potential way of introducing useful functionality into the final drug.

Section 4.9 introduces studies aimed at evaluating the possibility of adding a trigger to the prodrug. Incorporation of a suitable R" substituent, which could lead to remote activation of the drug, is described.

4.4 Diels-Alder Chemistry of 1-Methylsulfanyl-isobenzofuran

This section describes the Diels-Alder chemistry of 1-methylsulfanyl-isobenzofuran, i.e. the case where the R substituent is hydrogen, with simple dienophiles. These studies focused on dimethyl fumarate, dimethyl acetylenedicarboxylate, and *N*-phenyl maleimide. It also describes the chemistry of the resulting Diels-Alder adducts.

The first Diels-Alder reaction under consideration is that with the alkyne dienophile dimethyl acetylenedicarboxylate. 1-Methylsulfanyl-isobenzofuran **4.16** was prepared *in situ* as described in Section 4.2, and reacted with dimethyl acetylenedicarboxylate as shown in Fig. 4.4.1.

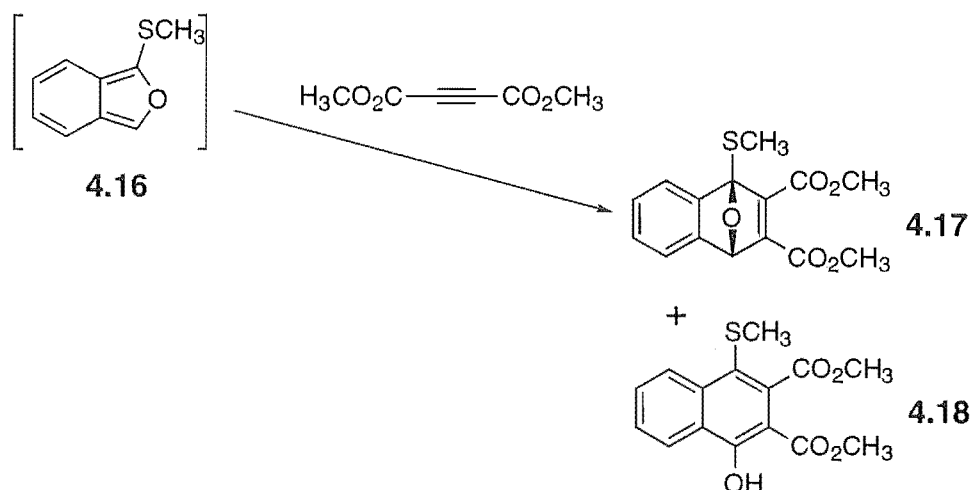


Fig. 4.4.1

Two products of interest were observed in this reaction; the Diels-Alder adduct itself **4.17**, in 25% yield, and the naphthalene product **4.18**, in 10% yield, formed via bridge cleavage of **4.17**. This result was in accord with the results of Bailey *et al.*⁶⁷ These two products could easily be identified and distinguished using mass spectrometry techniques, with the Diels-Alder adduct **4.17** giving rise to ions corresponding to retro-Diels-Alder fragmentation. Bridge cleavage of **4.17** to **4.18**, an acid-catalysed process, likely occurred during the isolation procedure, which involved radial silica chromatography.

An analogous Diels-Alder reaction was carried out using the alkene dienophile dimethyl fumarate, as shown in Fig. 4.4.2.

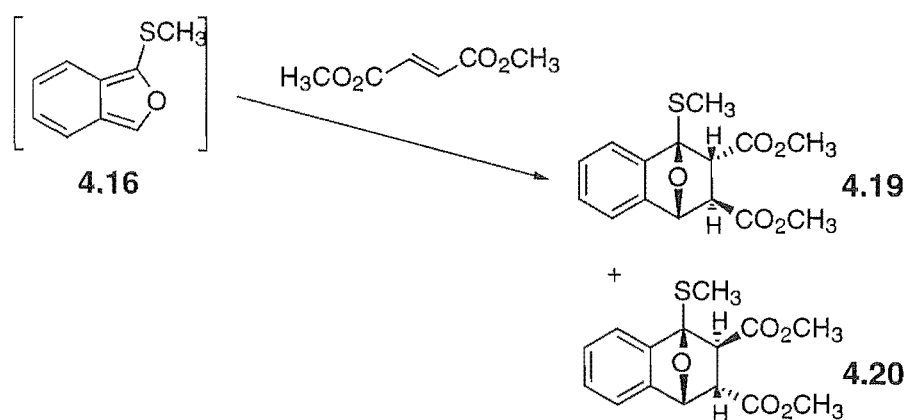


Fig. 4.4.2

In this case only bridged adducts were observed: **4.19** and **4.20**, which are stereoisomers of each other, isolated in a combined yield of 57%. No attempt was made to separate the isomers, and assignment of the stereochemistry, as shown in Fig. 4.4.2, was made by analogy with work done by Bailey *et al.*⁶⁷ In the ^1H NMR spectra, the major isomer **4.19** gave rise to characteristic doublets, corresponding to the protons α to the esters, at 3.21 and 3.79ppm, compared to corresponding doublets at 3.10 and 4.15ppm for the minor isomer **4.20**. The mass spectrum of this mixture included a peak at 308 mass units corresponding to the parent ion, as well as peaks corresponding to retro Diels-Alder fragmentation.

Bridge cleavage of the isomeric mixture of **4.19** and **4.20** was achieved by reaction with trifluoroacetic acid (TFA) as outlined in Fig. 4.4.3.

This resulted in the formation of two isomeric products: **4.21a** and **4.21b**, isolated in a combined yield of 82%. The mass spectrum of **4.21** also included a peak at 308 mass units corresponding to the parent ion, as well as a peak at 290 mass units corresponding to the loss of water. Notably, there were no peaks corresponding to retro Diels-Alder fragmentation, in accordance with bridge cleavage.

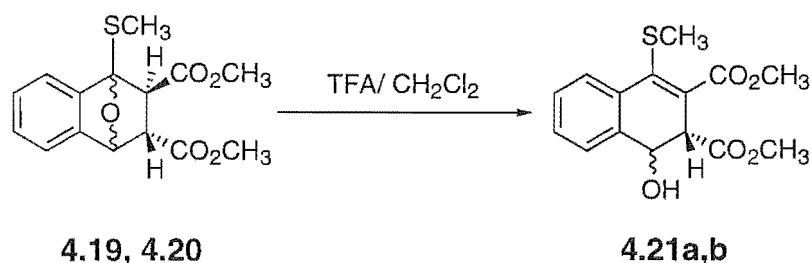


Fig. 4.4.3

The mechanism of such an acid-catalysed bridge cleavage is thought to be initiated by protonation of the bridge oxygen atom as shown in Fig 4.4.4. Subsequent elimination leads to the observed products **4.21a** and **4.21b**.^{29,70}

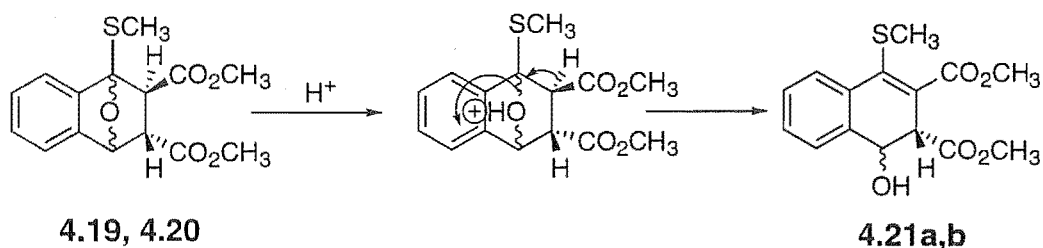


Fig. 4.4.4 Proposed mechanism of bridge cleavage.

Such acid-catalysed bridge cleavage mimics the first stage of the proposed *in vivo* prodrug activation pathway. Although not observed in this case, further loss of H₂O to give the corresponding planar aromatic compound was frequently observed in subsequent bridge cleavage reactions.

The third simple dienophile reacted with 1-methylsulfanyl-isobenzofuran **4.16** was *N*-phenyl maleimide, as shown in Fig. 4.4.5.

In this instance, none of the desired product **4.22** was isolated. This is likely due to the relative instability of 1-methylsulfanyl-isobenzofuran **4.16** under the reaction conditions. This relatively low stability makes **4.16** unsuitable for more complex Diels-Alder chemistry. The following section will describe the use of substitution at the 3-position of methylsulfanyl-isobenzofurans to enhance diene stability.

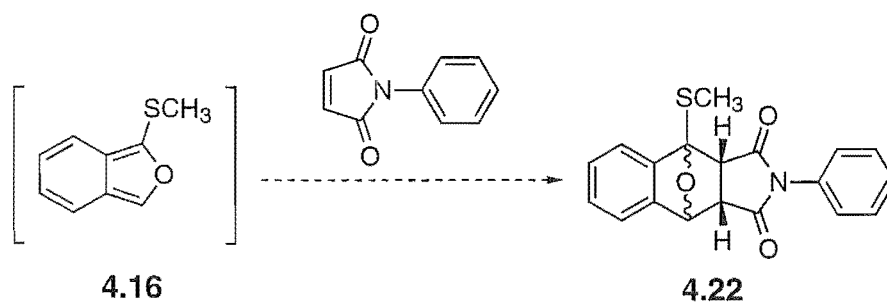


Fig. 4.4.5

4.5 Substitution at the 3 Position

Section 4.4 illustrated possible limitations in the Diels-Alder chemistry of isobenzofurans lacking a substituent at the 3-position. It is known that 3-alkyl- and 3-aryl-substituted dienes undergo Diels-Alder chemistry with dienophiles such as dimethyl fumarate more efficiently.⁶³ We were mainly interested in Diels-Alder chemistry involving *N*-aryl maleimides as the dienophile, which had failed with the unsubstituted case. Therefore this section describes the Diels-Alder chemistry of 3-alkyl and 3-aryl substituted dienes, along with the chemistry of the resulting adducts.

1-Methylsulfanyl-3-phenyl-isobenzofuran **4.23**, 1-methylsulfanyl-3-methyl-isobenzofuran **4.24**, and 1-methylsulfanyl-3-ethyl-isobenzofuran **4.25** were used as the dienes in a series of Diels-Alder reactions with *N*-phenyl maleimide as the dienophile, as outlined in Fig. 4.5.1.

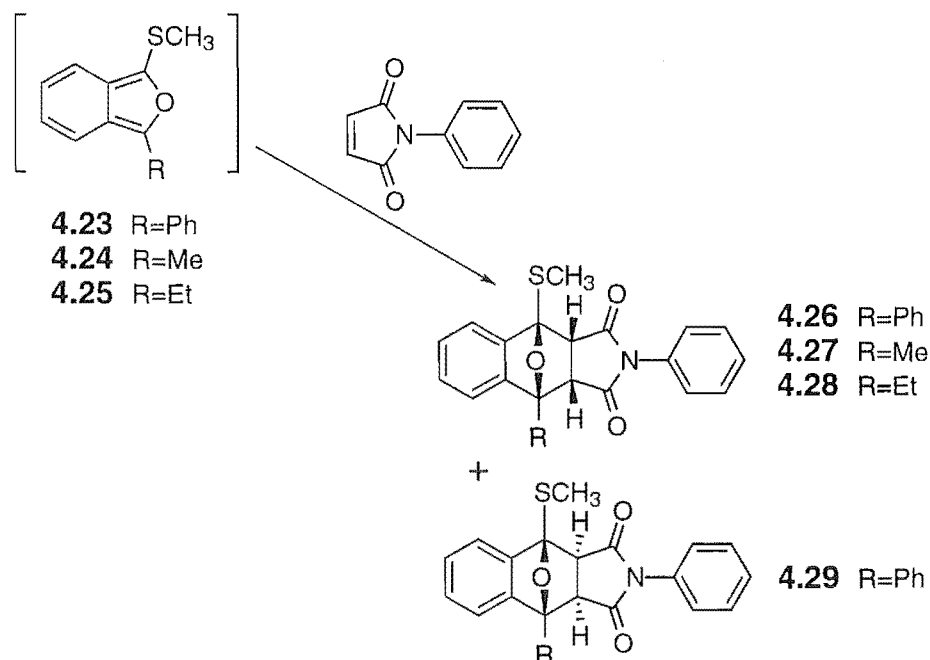


Fig. 4.5.1

With the phenyl-substituted diene, two products were isolated, **4.26** and **4.29**. The major isomer **4.26** was isolated in 48% yield and the minor isomer **4.29** in 1% yield. Based on analogy with related Diels-Alder adducts, which have been well characterised by x-ray crystallographic and ^1H NMR spectroscopic studies, the major product was identified as the *endo* isomer and the minor product as the *exo* isomer.⁶⁷ A notable feature in the ^1H NMR spectra of bridged Diels-Alder adducts such as these is the presence of two doublet resonances corresponding to the bridgehead protons. For the *endo* isomer **4.26** these two resonances were at 3.91 and 4.13ppm, while for the *exo* isomer **4.29** they were at 3.34 and 3.59ppm.

The presence of a phenyl group at the 3-position of the isobenzofuran had a beneficial effect on the reaction of the isobenzofuran with *N*-phenyl maleimide. However, being a large, bulky group it could sterically hinder intercalation into DNA. So the question of whether smaller groups, such as small alkyl groups, could also have such a positive effect was explored in the case of the methyl- and ethyl-substituted dienes.

The Diels-Alder reactions of 1-methylsulfanyl-3-methyl-isobenzofuran **4.24**, and 1-methylsulfanyl-3-ethyl-isobenzofuran **4.25** with *N*-phenyl maleimide gave the Diels-Alder adducts **4.27** and **4.28** respectively. In both instances only one stereoisomer was observed as the product, which was identified, by analogy with the phenyl substituted case, as the *endo* isomer. The methyl-substituted adduct **4.27** contained doublets in the ^1H NMR spectrum corresponding to the bridgehead protons at 3.67 and 3.82ppm, while for the ethyl-substituted adduct **4.28**, doublets were at 3.72 and 3.79ppm.

In the case of the methyl-substituted adduct **4.27**, the assignment of an *endo* configuration was confirmed by X-ray crystallographic studies (cf. Appendix).

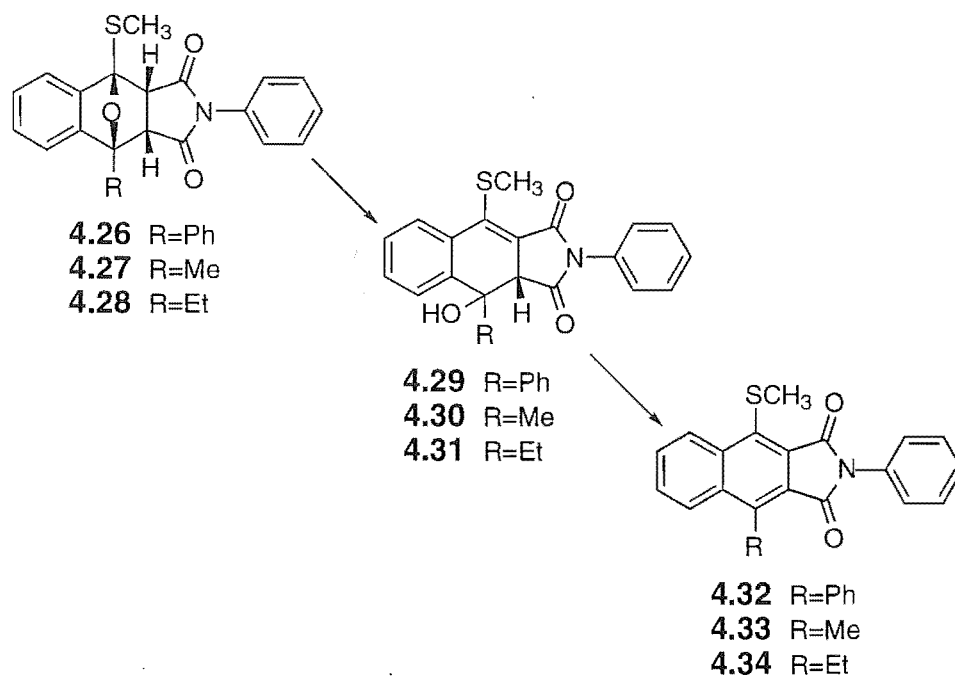


Fig. 4.5.2

The bridge cleavage and aromatisation reactions of the Diels-Alder adducts **4.26**, **4.27**, and **4.28** are outlined in Fig. 4.5.2. It was expected that reaction of the phenyl-substituted *endo* adduct, **4.26**, with TFA would lead not only to bridge cleavage, but also directly to the aromatisation product **4.32**, through spontaneous dehydration. However in fact, the intermediate product **4.29** was observed, easily identifiable by ^1H NMR spectroscopy due to the loss of the two doublets at 3.91 and 4.13ppm, along with the observation of a new singlet resonance at 4.22ppm corresponding to the remaining lone hydrogen of the ring junction. When purification of **4.29** was attempted by radial silica chromatography it was found to decompose on the silica to the originally expected product **4.32**, as identified by the loss of the 4.22ppm singlet from the ^1H spectrum. Interestingly, the intermediate and final products were indistinguishable by mass spectrometry, with a peak at 395 mass units corresponding to loss of water in **4.29**, and the parent ion in **4.32**.

A similar sequence was observed in the aromatisation of the methyl-substituted adduct **4.27**. Reaction with TFA led to the intermediate product **4.30**. However in this instance **4.30** was purified successfully by radial silica chromatography without evident dehydration. Subsequent dissolution in acetonitrile and addition of concentrated hydrochloric acid resulted in aromatisation, giving the desired product **4.33**.

In the light of the above observations, the ethyl substituted adduct **4.28** was aromatised by a more direct route. This involved treatment of **4.28** directly with concentrated hydrochloric acid to give the aromatised product **4.34**.

Diels-Alder reactions of this type were observed to work more efficiently when an alkyl or aryl group was introduced at the 3-position of the isobenzofuran. Diels-Alder adducts so formed were able to be converted, through acid-catalysed bridge cleavage and dehydration, to their corresponding planar, aromatic forms.

An example of incorporating a leaving group at the 3-position of these isobenzofurans is described in the following section.

4.6 'R' as a Leaving Group

The introduction of a leaving group into such Diels-Alder adducts offers the possibility of an alternative route to aromatisation.²⁹ The phenylsulfanyl group was chosen as the leaving group for this study. Fig. 4.6.1 shows how this was incorporated into a Diels-Alder adduct via the isobenzofuran diene.

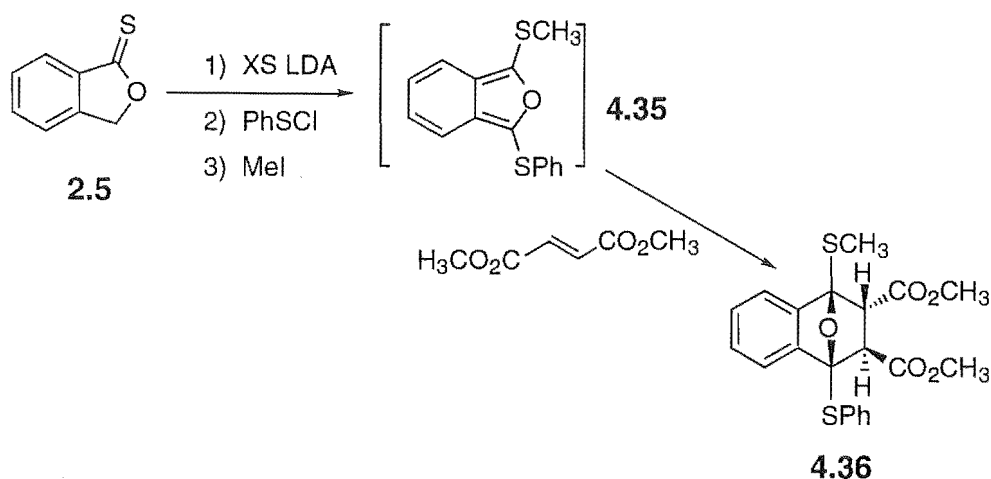


Fig. 4.6.1

The *in situ* preparation of the isobenzofuran **4.35** from its precursor **2.5** was carried out in the presence of an excess of LDA. This deprotonates the thione at the 3 position and lithiates the furan ring. Reaction with phenylsulfenyl chloride followed by alkylation thus gave rise to the disulfanyl-substituted isobenzofuran **4.35**. This was immediately trapped with dimethyl fumarate in Diels-Alder fashion to give the adduct **4.36** in one isomeric form, identified as the 9-*exo*-10-*endo* isomer by analogy with related adducts.⁶⁷ This adduct was characterised in its mass spectrum by a peak at 416 mass units corresponding to the parent ion, as well as a peak at 307 mass units corresponding to the loss of the phenylsulfanyl group.

However, the modifications made to this reaction sequence resulted in lowering the yield of the Diels-Alder adduct from 57%, in the 3-hydrogen-substituted case, to below 1%. This substantial drop in efficiency makes the route unsuitable for more complex Diels-Alder chemistry and so these studies were not pursued further.

4.7 Extending the Ring System

Optimum intercalation of an intercalating agent requires the presence of three or more fused coplanar rings. The Diels-Alder adducts and their corresponding aromatised products considered thus far have been designed with a tricyclic chromophore in mind. This section explores the possibility of varying the R' substituent so as to extend the system to four rings (cf. Fig. 4.3.1).

The isonaphthofuranthiones **2.22** and **2.25** were treated with LDA and methyl iodide, as shown in Fig. 4.7.1, to prepare the tricyclic dienes, **4.37** and **4.38** respectively, *in situ*.

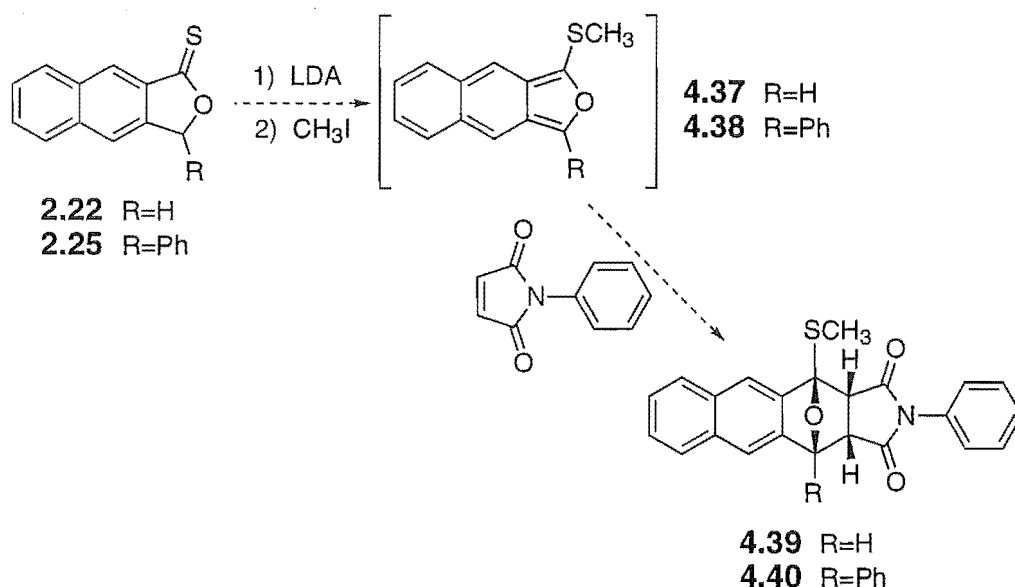


Fig. 4.7.1

Since the isonaphthofurans **4.37** and **4.38** are less aromatic in nature than the corresponding isobenzofurans, they would be expected to be more reactive than the corresponding isobenzofurans. Attempts to trap **4.37** or **4.38** with the dienophile *N*-phenyl maleimide however, were unsuccessful. Neither of the desired Diels-Alder adducts, **4.39** or **4.40** respectively, could be isolated.

A similar result was observed when the quinoline-containing furanthione **2.34** was used as the starting material as outlined in Fig. 4.7.2.

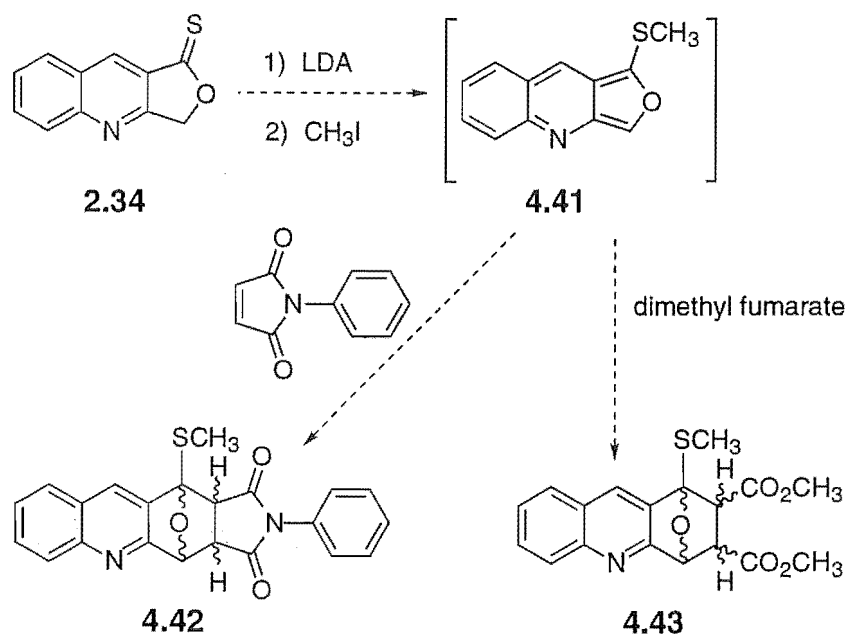


Fig. 4.7.2

Attempts were made to trap the intermediate **4.41** with *N*-phenyl maleimide and dimethyl fumarate. However, again, neither of the desired Diels-Alder adducts, **4.42** or **4.43** respectively, were isolated.

Consideration of these results caused us to refocus on adducts which would lead to a tricyclic chromophore.

4.8 Modification of the Dienophile

The survey of isobenzofurans and derivatives described in previous sections has shown the viability of their Diels-Alder chemistry with simple dienophiles. Attention is now focused on modification of the dienophile, particularly the possibility of incorporating nitrogen-based substituents. Nitrogen-based substituents may allow tuning of the biological activity of the products. Variations

in the dienophile were explored by adding functionality into the Diels-Alder adducts via the electron withdrawing groups (EWG). This section initially discusses the Diels-Alder reactions of fumaramides (4.8.1). The Diels-Alder reactions of *N*-aryl maleimides, and the chemistry of the resulting adducts, are then described (4.8.2).

4.8.1 Fumaramides

The first class of nitrogen-enriched dienophiles investigated was amide derivatives of fumaric acid. Chapter 3 described the synthesis of two such compounds; the fumaramides **3.5** and **3.6**.

The Diels-Alder reactions of 1-methylsulfanyl-isobenzofuran **4.16**, and 1-methylsulfanyl-3-phenyl-isobenzofuran **4.23** with **3.5** are shown in Fig. 4.8.1.

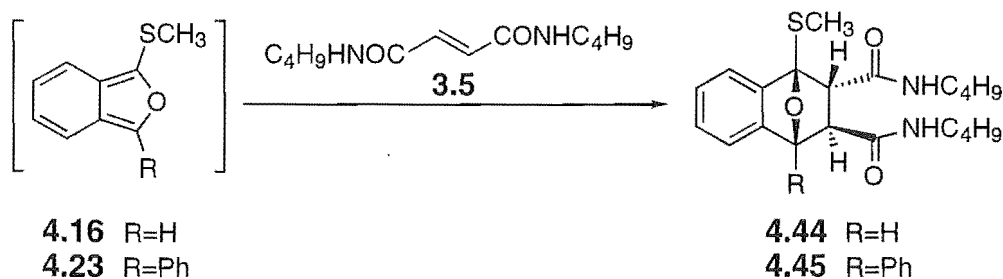


Fig. 4.8.1

This resulted in the formation of the Diels-Alder adducts **4.44** and **4.45** respectively, each of which was isolated in one isomeric form only, identified by analogy with related adducts.⁶⁷ Adduct **4.44** was isolated in 20% yield, and **4.45** in 25% yield. Both adducts gave rise to a characteristic pair of doublets in the ¹H NMR spectra; at 2.75 and 2.94ppm for **4.44**, and 3.36 and 3.66ppm for **4.45**. The mass spectra for these compounds showed peaks corresponding to the parent ions, at 389 mass units for **4.44**, and 466 mass units for **4.45**.

1-Methylsulfanyl-isobenzofuran **4.16**, and 1-methylsulfanyl-3-phenyl-isobenzofuran **4.23** were likewise reacted in Diels-Alder fashion with the dienophile **3.6** as shown in Fig. 4.8.2. In neither of these cases could the expected Diels-Alder adducts, **4.46** or **4.47**, be isolated.

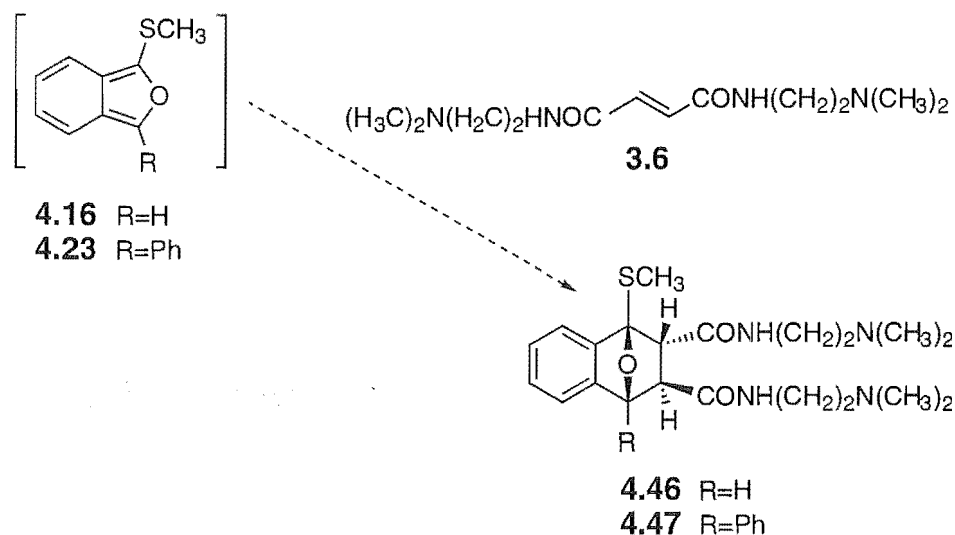


Fig. 4.8.2

There was a lack of success in introducing further functionality into such amides and so these studies were not pursued further.

4.8.2 *N*-Aryl maleimides

N-Phenyl maleimide had proved to be a very effective dienophile for reaction with 3-substituted isobenzofurans. The possibility of using *N*-aryl maleimides to introduce additional functionality into our Diels-Alder adducts was thus explored further. In particular we were interested in introducing a range of nitrogen-based substituents at the *ortho* and *meta* positions of the phenyl ring. Substituents at these positions can provide the base for functional groups to interact with the grooves of DNA (cf. Fig. 5.1.1).

An exploratory Diels-Alder reaction, performed using a nitro-derivative of *N*-phenyl maleimide, is shown in Fig. 4.8.3. 1-Methylsulfanyl-3-phenyl-isobenzofuran **4.23** was here trapped with *N*-(3-nitrophenyl) maleimide **3.23** to give the adduct **4.48**. Only the *endo* stereoisomer was isolated. The ^1H NMR spectrum of **4.48** contained resonances at 4.00 and 4.25ppm, corresponding to the bridgehead protons. Its mass spectrum was characterised by a peak at 458 mass units, corresponding to the parent ion, and a peak at 412 mass units, corresponding to loss of the nitro group.

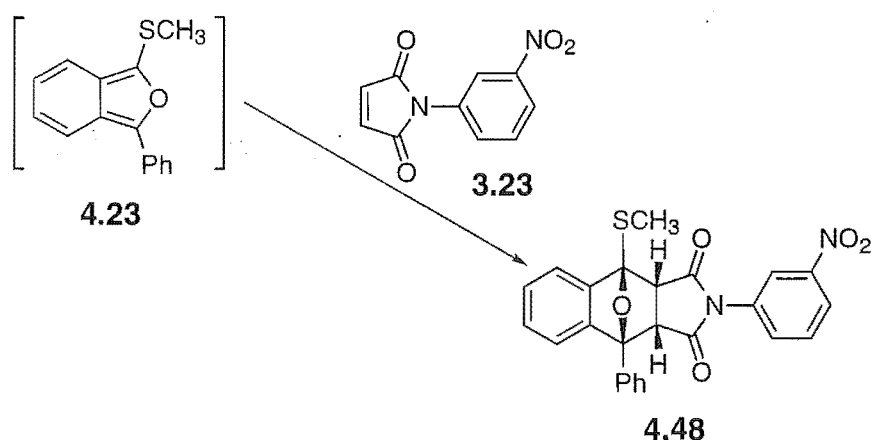


Fig. 4.8.3

The successful formation of **4.48** is encouraging for the synthesis of substituted Diels-Alder adducts. It would be possible to modify the nitro-substituent of **4.48** to generate functional groups capable of binding in the groove of DNA. Instead though, we decided to explore Diels-Alder reactions with alternative functionalised *N*-aryl maleimides, as they open up a more convergent strategy for the synthesis of complex Diels-Alder adducts. This avenue of research was evaluated by introducing amide substituents to the *N*-phenyl maleimide skeleton, at either the *ortho* or *meta* position.

The *ortho*-substituted *N*-phenyl maleimide derivative **3.29** was prepared as described as in Chapter 3 and used to trap both 1-methylsulfanyl-3-phenyl-

isobenzofuran **4.23** and 1-methylsulfanyl-3-methyl-isobenzofuran **4.24** as shown in Fig. 4.8.4. In each case, isomeric pairs of products, **4.49**, **4.51** and **4.50**, **4.52** respectively, were obtained (cf. Fig. 4.8.6).

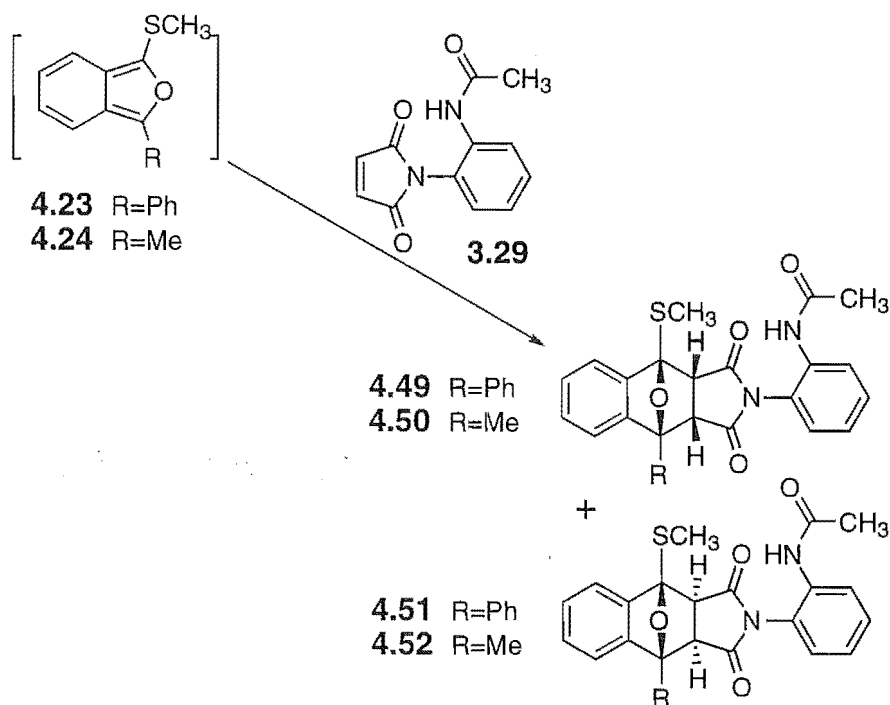


Fig. 4.8.4

Likewise, the *meta*-substituted *N*-phenyl maleimide derivative **3.28** was used to trap both 1-methylsulfanyl-3-phenyl-isobenzofuran **4.23** and 1-methylsulfanyl-3-methyl-isobenzofuran **4.24** as shown in Fig. 4.8.5. Once again, isomeric pairs of products, **4.53**, **4.55** and **4.54**, **4.56** respectively, were obtained (cf. Fig. 4.8.6).

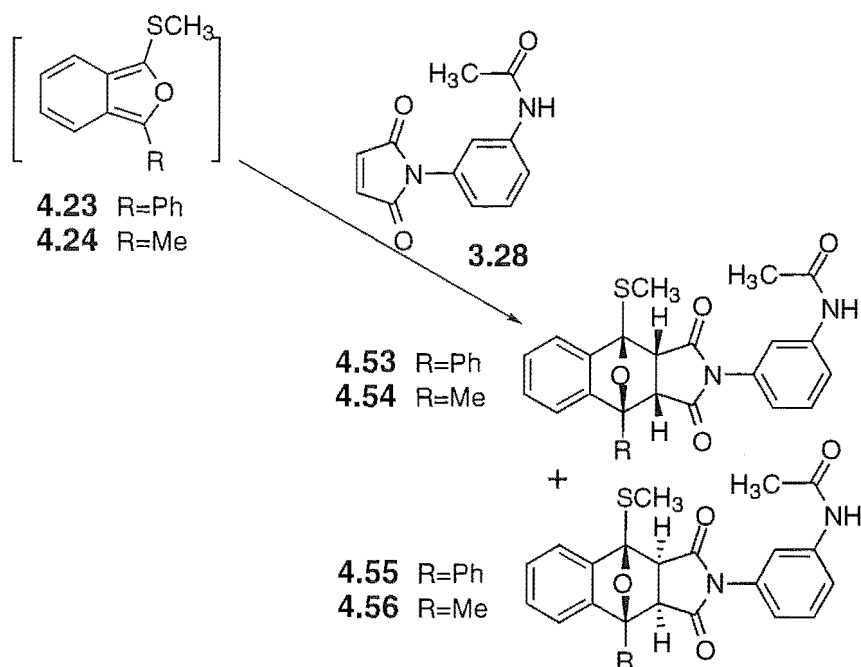


Fig. 4.8.5

The mass spectra for this series of *ortho* and *meta* acetamido-substituted Diels-Alder adducts were characterised by the presence of a parent ion, at 470 mass units in the case of 3-phenyl-substitution and 408 mass units in the case of 3-methyl-substitution, along with an ion at 42 mass units lower, corresponding to loss of the acyl group. Their ^1H NMR spectra were characterised by a pair of doublet resonances at around 4ppm corresponding to the two bridgehead protons.

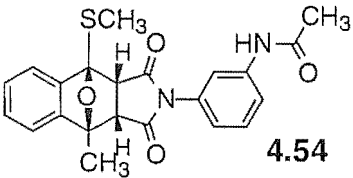
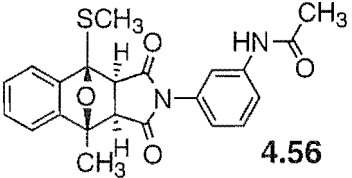
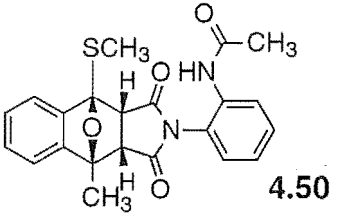
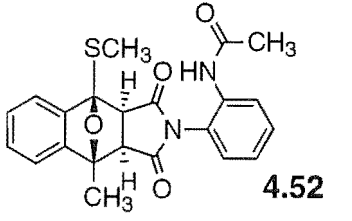
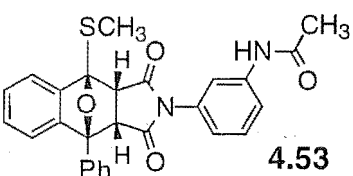
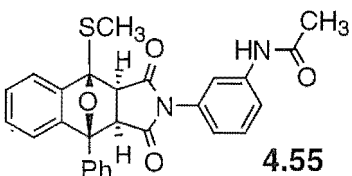
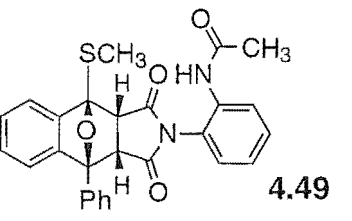
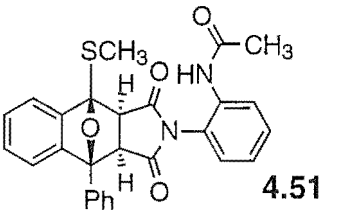
<u>Diels-Alder Adducts</u>		<u>Isomeric Ratio</u> (Combined % Yield)
 4.54	 4.56	2.4 : 1 (64%)
 4.50	 4.52	24 : 1 (27%)
 4.53	 4.55	43 : 1 (13%)
 4.49	 4.51	11 : 1 (14%)

Fig. 4.8.6

Fig. 4.8.6 shows the isomeric ratios obtained for this series of reactions, along with the combined percentage yields for both isomers. In each case the *endo* isomer predominated over the *exo*, although the diastereoselectivity showed substantial variation.

4.8.3 Bridge Cleavage

The acetamido-substituted Diels-Alder adducts described in Section 4.8.2 were bridge cleaved and aromatised directly, by dissolving in acetonitrile, adding concentrated hydrochloric acid, and stirring at room temperature.

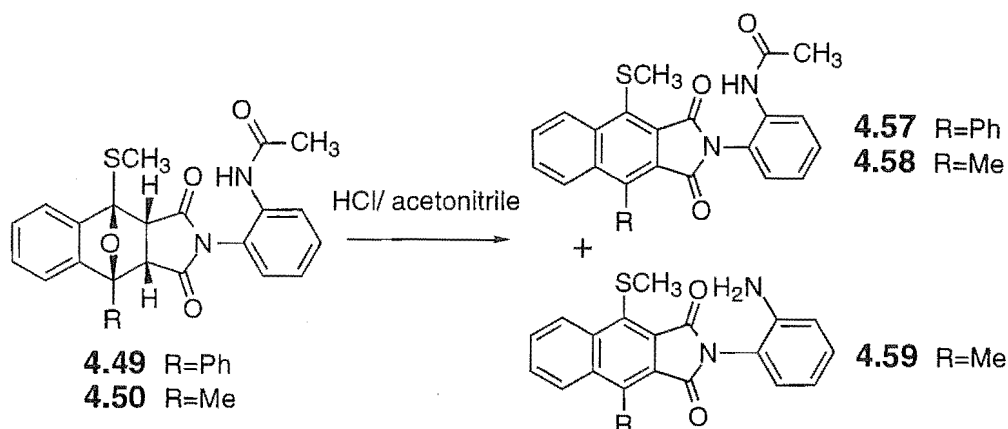


Fig. 4.8.7

Bridge cleavage of the *ortho*-substituted adducts is shown in Fig. 4.8.7. In the phenyl case, which was stirred for two days, only one product was observed: the expected aromatic product **4.57**. However, upon the extended acid treatment required in the methyl case (five days), two products were observed, the expected aromatic product **4.58** and the additional aromatic compound **4.59**, where the amide had been hydrolysed to give an amine. The ¹H NMR spectrum of **4.58** contained a resonance at 2.08ppm corresponding to the acetamido-methyl group. This resonance was absent in the ¹H NMR spectrum of **4.59**. The mass spectrum of **4.58** was characterised by a peak at 390 mass units, corresponding to the parent ion, and another peak at 348 mass units, corresponding to loss of the acetyl group. In the case of **4.59**, the mass spectrum also contained a peak at 348 mass units, in this case corresponding to the parent ion.

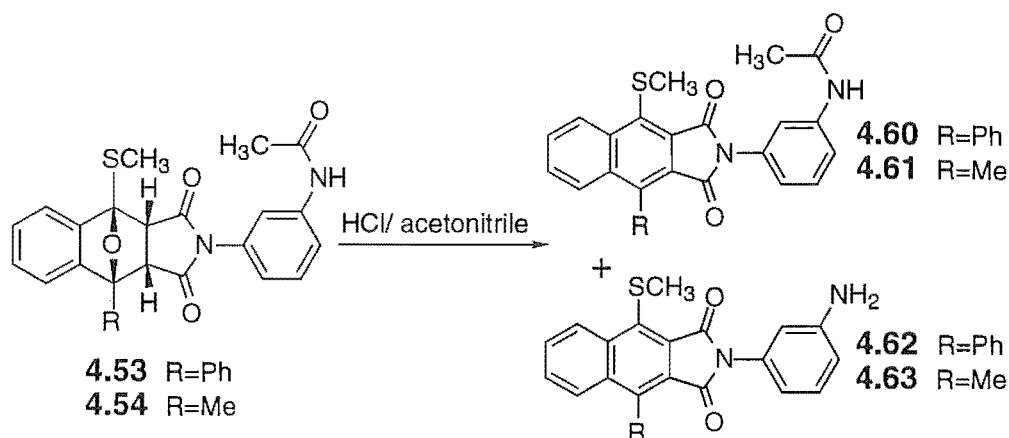


Fig. 4.8.8

In the *meta*-substituted cases, amide hydrolysis competed with aromatisation in each case, as shown in Fig. 4.8.8. The pairs of products were: **4.60** and **4.62** for the phenyl case, and **4.61** and **4.63** for the methyl case. These corresponded to the expected aromatic product and the amine derivative, formed via amide hydrolysis. The ¹H NMR spectra of the acetamido-substituted products, **4.60** and **4.61**, each contained a resonance corresponding to the acetamido methyl group, at 2.15 and 2.18ppm respectively. These resonances were absent in the corresponding ¹H NMR spectra of **4.62** and **4.63**, indicative of amide hydrolysis. The mass spectra of **4.60** and **4.61** contained peaks corresponding to the parent ions, at 452 and 390 mass units respectively, along with peaks at 410 and 348 mass units respectively, corresponding to loss of the acetyl group. The mass spectra of **4.62** and **4.63** also contained peaks at 410 and 348 mass units respectively, in this case corresponding to the parent ions.

This work has demonstrated the viability of synthesising Diels-Alder adducts from amide derivatives of *N*-phenyl maleimide. Extension of this work into more highly functionalised derivatives is covered in Chapter 5.

4.9 Addition of a Trigger

Having established that functional groups could be introduced into Diels-Alder adducts, it was decided to evaluate whether aromatisation could be triggered remotely by incorporation of a suitable sulfur-based substituent. This section, therefore, explores the possibility of adding a trigger to our Diels-Alder adducts by variation of R" (cf. Fig. 4.3.1). The term 'trigger' refers, in this case, to a chemical moiety which can induce bridge cleavage.

An example of such a trigger would be a group which allowed the non-planar bridged adduct to be selectively converted to the corresponding planar form in the reducing conditions of an hypoxic tumour cell. One such bioreductive trigger is shown in Fig. 4.9.1.

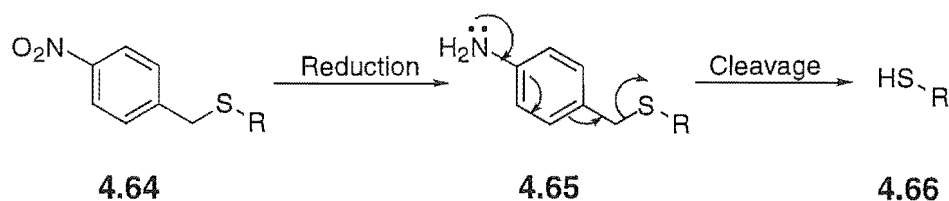


Fig. 4.9.1

Reduction of the nitro moiety in the prodrug **4.64** to an amine moiety in **4.65** leads to cleavage of the sulfur-carbon bond to give **4.66** as shown, with subsequent conversion of the compound to its active drug form.

The model trigger chosen for this work was an analogue of this process. A benzyl acetate moiety was introduced into the Diels-Alder adduct via 4-chloromethylphenyl acetate.

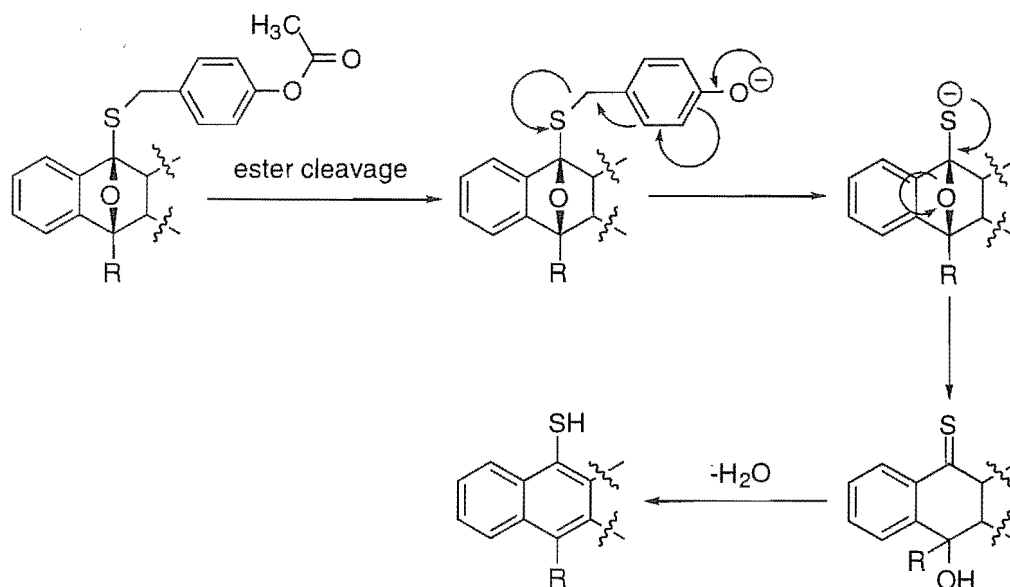


Fig. 4.9.2 Proposed mode of activation

The basic mode of activation proposed for this class of compound is outlined in Fig. 4.9.2. Cleavage of the ester leads to cleavage of the carbon-sulfur bond through a quinomethide intermediate. The thiolate anion thus produced is unstable and collapses to give the active planar aromatic compound.⁷¹

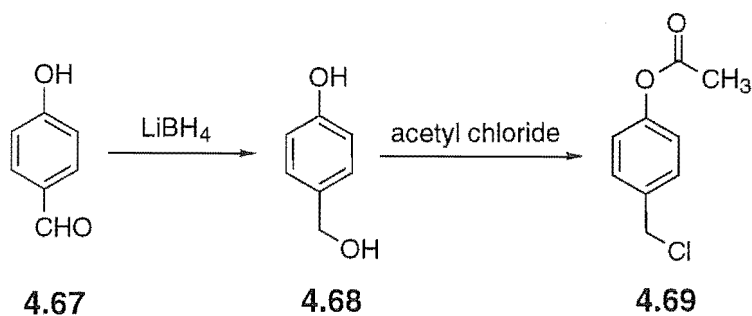


Fig. 4.9.3

4-Chloromethylphenyl acetate **4.69** was synthesised as the precursor to such a trigger, as shown in Fig. 4.9.3. Reduction of 4-hydroxybenzaldehyde **4.67** using lithium borohydride gave 4-hydroxybenzyl alcohol **4.68**.²⁹ Reaction with acetyl

chloride converted **4.68** to 4-chloromethylphenyl acetate **4.69**, which was purified by distillation under reduced pressure.⁷²

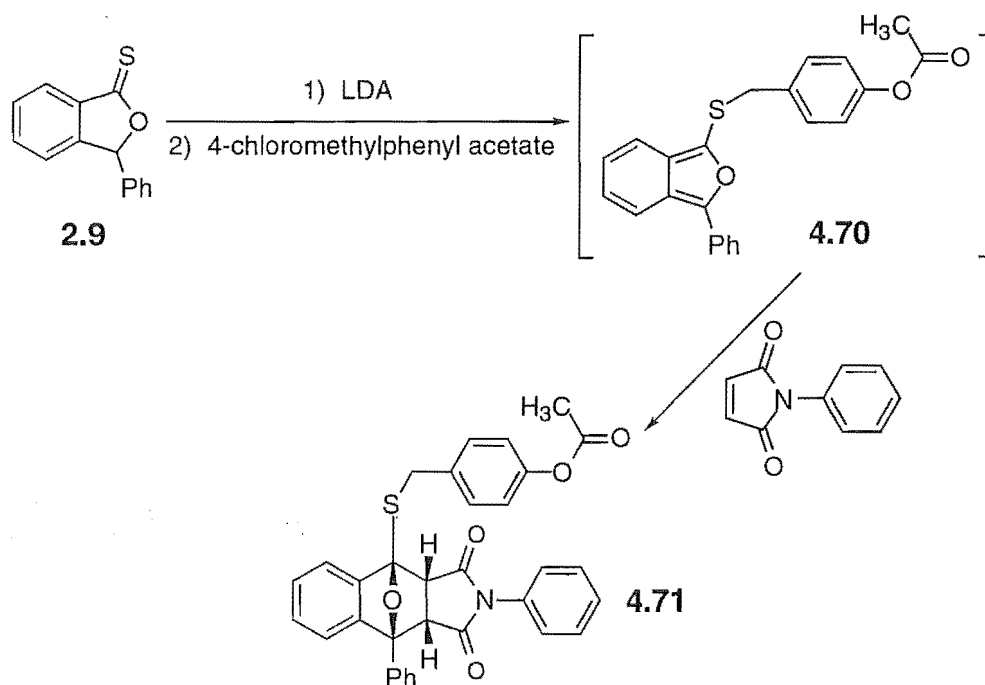


Fig. 4.9.4

This triggering moiety was introduced into a Diels-Alder adduct as shown in Fig. 4.9.4. 3-Phenyl-1(3H)-isobenzofuranthione **2.9** was deprotonated with LDA and alkylated with 4-chloromethylphenyl acetate to generate the isobenzofuran **4.70**. This was trapped *in situ* with *N*-phenyl maleimide to give the desired Diels-Alder adduct **4.71**, as a single diastereoisomer. This was identified as the *endo* isomer by analogy with related adducts. The ¹H NMR spectrum of **4.71** showed characteristic doublet resonances at 3.96 and 4.16ppm corresponding to the bridgehead protons. It also contained a characteristic singlet resonance at 2.30ppm, corresponding to the methyl group. The mass spectrum of **4.71** was characterised by a peak at 547 mass units, corresponding to the parent ion.

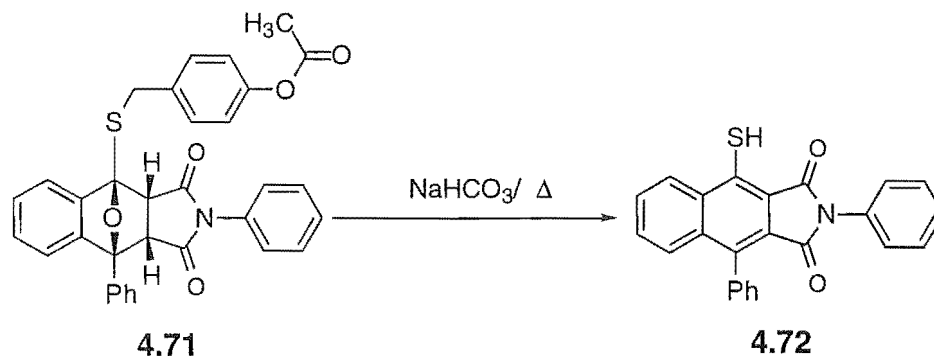


Fig. 4.9.5

Bridge cleavage of **4.71** was performed by base hydrolysis as shown in Fig. 4.9.5. The Diels-Alder adduct **4.71** was refluxed in methanol with sodium bicarbonate. This resulted in cleavage of the bridge and subsequent dehydration to give the aromatic product **4.72**. No intermediate product was observed. The ^1H NMR spectrum of **4.72** was characterised by the absence of the two bridgehead proton doublet resonances, at 3.96 and 4.16ppm, found for **4.71**. The singlet resonance at 2.30ppm, characteristic of **4.71**, was also absent. The mass spectrum of **4.72** was characterised by a peak at 381 mass units, corresponding to the parent ion.

It was thus demonstrated that remote activation of such a system can lead to aromatisation, as required for our prodrug strategy.

4.10 Summary

A wide range of Diels-Alder chemistry was described in this chapter. 1-Methylsulfanyl isobenzofuran was reacted with a range of simple dienophiles, in particular dimethyl fumarate, dimethyl acetylenedicarboxylate, and *N*-phenyl maleimide. This isobenzofuran proved unsuitable for use with *N*-aryl maleimides, the dienophiles under particular scrutiny in this thesis. Therefore the Diels-Alder

chemistry of 3-substituted isobenzofurans with *N*-phenyl maleimide was explored, resulting successfully in a range of adducts. The introduction of a leaving group into such adducts was also demonstrated, offering the possibility of an additional pathway to aromatisation. When the ring system of the diene was extended, no Diels-Alder adducts could be isolated.

The Diels-Alder chemistry of functionalised dienophiles was also described. Diels-Alder adducts were successfully synthesised from the reaction of fumaramides with isobenzofurans. It was demonstrated that functionality could be introduced into Diels-Alder adducts via substituents on the phenyl ring of *N*-aryl maleimides. It was also demonstrated that aromatisation of Diels-Alder adducts could be triggered remotely by the introduction of a suitable sulfur-based substituent.

*DIELS-ALDER APPROACHES
TO ANTI-CANCER PRODRUGS*

**Part A:
Model Investigations**

Conclusion

A model system for the synthesis of Diels-Alder adducts as prodrugs for anti-cancer agents was successfully developed by the systematic variation of adduct substituents. This system was successful for a range of isobenzofurans, and was flexible, allowing the introduction of a range of functionality via the dienophile. However, in some cases the limited stability of the oxygen bridge presented difficulties. There was also the need to introduce biological functionality into these adducts. Part B addresses these issues.

*DIELS-ALDER APPROACHES
TO ANTI-CANCER PRODRUGS*

**Part B:
Towards Biologically
Active Prodrugs**

Introduction

Based on the model system developed in Part A, Part B describes the extension of this system towards biologically active prodrugs for anti-cancer agents. Chapter 5 describes the introduction of biological functionality into Diels-Alder adducts via the dienophile. Chapter 6 describes the introduction of a sulfur bridge into such adducts.

Chapter 5

OXYGEN-BRIDGED DIELS- ALDER ADDUCTS 2

5.1 Introduction

This chapter concerns the synthesis of dienophiles with functionality that could promote binding to DNA, either reversibly or irreversibly. The evaluation of the Diels-Alder reactivity of these dienophiles with isobenzofurans is then described.

For a prodrug of an intercalator to be efficiently transported throughout the body and permeate a solid tumour via diffusion, a low DNA binding affinity is important. This is counterbalanced with the need for the drug itself to have a high DNA binding affinity, thereby increasing its toxicity. The compounds in this chapter were designed to address both these factors. Our basic design concept was to incorporate further functionality into the model systems developed in Part A, leading to non-planar prodrugs with low DNA binding affinity, but where aromatised derivatives have the potential for high DNA binding affinity.

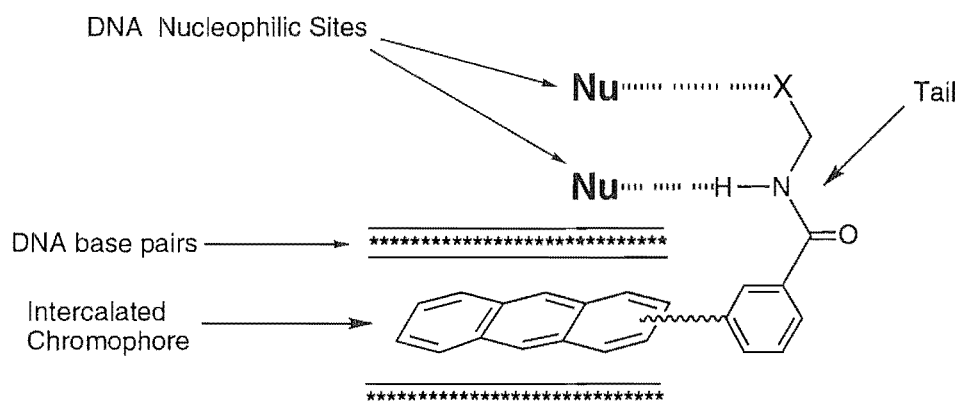


Fig. 5.1.1

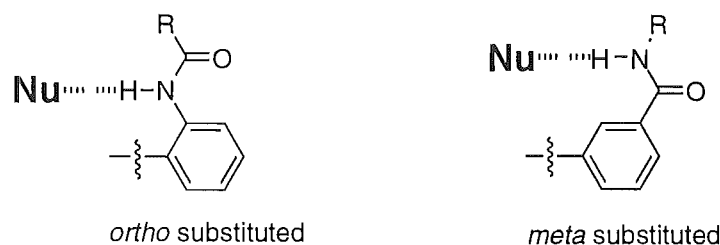
Fig. 5.1.1 illustrates this concept, showing diagrammatically the intercalation of a planar chromophore between base-pairs of DNA. At the same time the 'tail' of the intercalating agent is able to stabilise the intercalation through hydrogen-bonding, or alkylation, with nucleophilic sites in a groove of the DNA. DNA is rich in nucleophilic sites, such as the phosphate backbone or heteroatoms in the DNA base pairs.

Examples of functional groups (X) which could participate in hydrogen-bonding are shown in Fig. 5.1.2. The presence of ammonium substituents is important for the action of many intercalating agents.



Fig. 5.1.2

In *N*-aryl maleimides, the *ortho* and *meta* positions are both sites at which appropriately oriented electrophilic moieties could be incorporated via an amide bond. A schematic analysis of the geometries of these options is presented in Fig. 5.1.3.

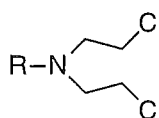
**Fig. 5.1.3**

Here it can be seen that a protonated nitrogen centre pointing back towards the DNA strand, and therefore potentially able to participate in hydrogen-bonding, can result from direct linkage of the amine at the *ortho* position, or via a carboxyl group at the *meta* position. These considerations were taken into account in the design and synthesis of the compounds presented in this chapter.

Intercalation with a nucleophilic site on DNA can be either non-covalent, as in the case of hydrogen-bonding, or covalent, as in the case of alkylating agents.

5.2 Addition of an Alkylating Group

In anti-tumour chemistry, the term 'alkylating agent' refers to a drug whose mode of action is the covalent bonding of an alkyl group to cellular molecules.⁷³ Such alkylating agents have played a vital role in the progress of cancer chemotherapy. In fact the nitrogen mustards were the first non-hormonal agents to show significant anti-tumour activity in humans.^{74,75,76} Fig. 5.2.1 shows the general structure of the nitrogen mustards.

**Fig. 5.2.1**

The proposed mechanism of alkylation by the nitrogen mustards is shown in Fig. 5.2.2.

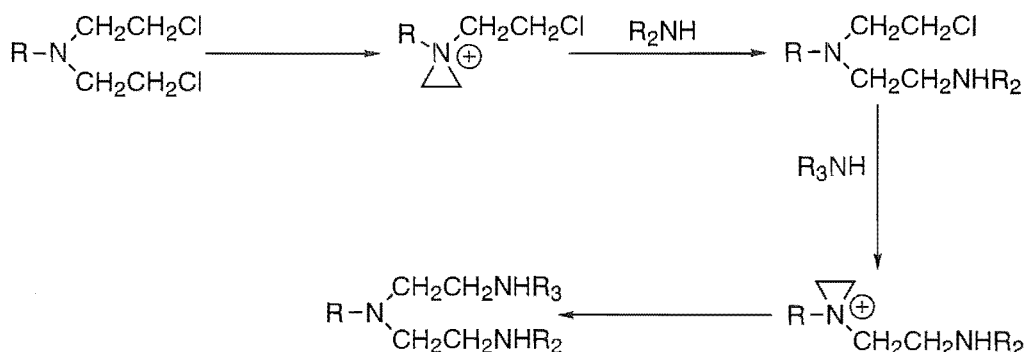


Fig. 5.2.2

The process is triggered by the initial loss of chloride, which results in the β -carbon reacting with the internal nitrogen atom, forming a cyclic, highly reactive aziridinium moiety. Such a moiety can react with a nucleophilic centre in DNA, resulting in alkylation. In the case of the nitrogen mustards, this process can be repeated, resulting in DNA cross-linking.

We wished to incorporate the potential for alkylation into our *N*-aryl maleimide based dienophile system, via an amide linker. Acylation of 2-nitroaniline **5.1** with chloroacetyl chloride and bromoacetyl bromide was carried out as shown in Fig. 5.2.3, to give **5.2** and **5.3** respectively.

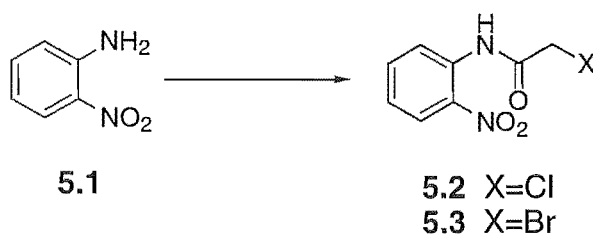


Fig. 5.2.3

Fig. 5.2.4 shows the similar reactions which were repeated starting with 3-nitroaniline **5.4** to give the products **5.5** and **5.6**. All these acylation reactions proceeded smoothly and in quantitative yield.

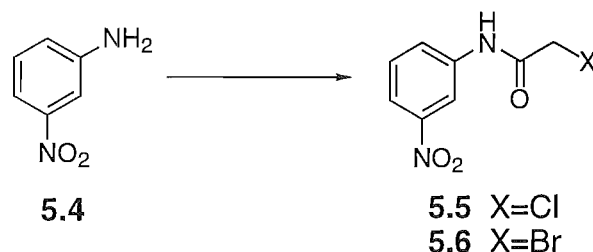


Fig. 5.2.4

Reduction of the nitro groups to amino groups proved complicated. This was attempted via hydrogenation, catalysed by palladium-on-carbon. This had been found to be effective for other nitrobenzene derivatives. In the bromo-substituted cases, **5.3** and **5.6**, these reducing conditions were found to result in loss of the bromo-substituent. In the case of **5.5** also, with a *meta*-substituted chloro-substituent, the desired product was not isolated. In this case two products were isolated. Each of these had undergone reductive cleavage of the carbon-halogen bond. They differed in that one product, **3.11**, retained an intact nitro group, whereas the other, **3.12**, was an amine. This reaction is shown in Fig. 5.2.5.

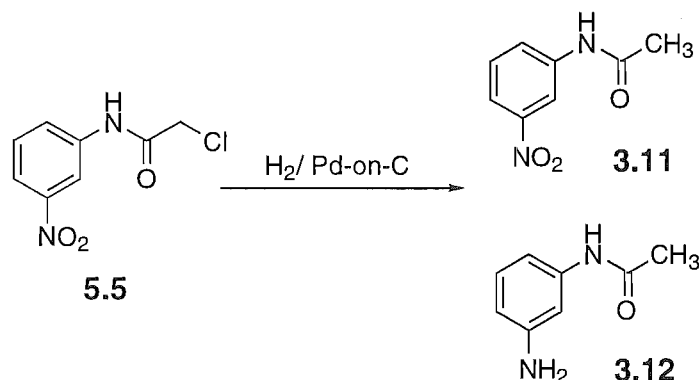


Fig. 5.2.5

The observed products, **3.11** and **3.12**, were easily identified as both compounds had been synthesised earlier. This product distribution showed that side-chain reduction was more facile than reduction of the nitro group in **5.5**.

Investigations into the *meta*-substituted cases were discontinued due to the complexities of reaction and the fact that this system is not optimally aligned for interaction with DNA.

In the case of the *ortho*-substituted **5.2** however the hydrogenation proceeded smoothly, forming one product only, the desired amine **5.7** as shown in Fig. 5.2.6.

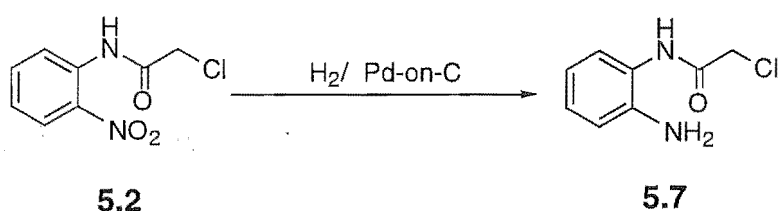


Fig. 5.2.6

As such amine compounds are known to decompose on prolonged storage, **5.7** was immediately used as the starting material in the reaction with maleic anhydride outlined in Fig. 5.2.7.

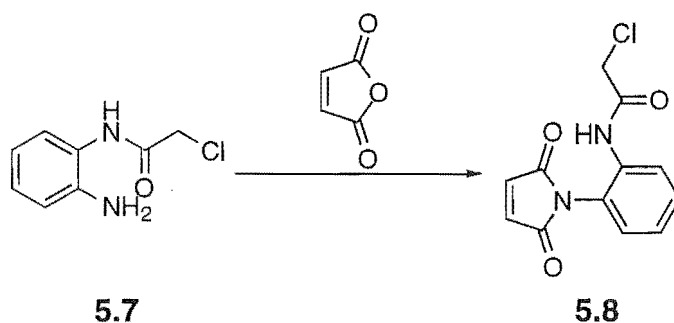


Fig. 5.2.7

This reaction, the introduction of an amine into the maleic skeleton, is analogous to the synthesis of *N*-phenyl maleimide as discussed earlier in this

thesis. The result was the desired product *N* - (2-chloroacetylaminophenyl)maleimide **5.8**, whose ^1H NMR spectrum had the characteristic sharp singlet at 6.94ppm corresponding to the two equivalent maleimide protons.

N-(2-chloroacetylaminophenyl)maleimide **5.8** was now available for use as a dienophile in further Diels-Alder chemistry.

5.3 Diels Alder Reactions of *N*-(2-chloroacetylaminophenyl)maleimide

This section describes the incorporation of a potential alkylating moiety into Diels-Alder adducts and the bridge cleavage of the resulting adducts.

5.3.1 Diels-Alder Reactions

N-(2-chloroacetylaminophenyl)maleimide **5.8** was used to trap 1-methylsulfanyl-3-phenyl-isobenzofuran **4.23** and 1-methylsulfanyl-3-methyl-isobenzofuran **4.24** in Diels-Alder reactions as outlined in Fig. 5.3.1. Both of these reactions were successful in generating Diels-Alder adducts. In each case only a single stereoisomer was isolated, **5.9** and **5.10** respectively. Both of these adducts were assigned as *endo* isomers by comparison with analogous adducts.

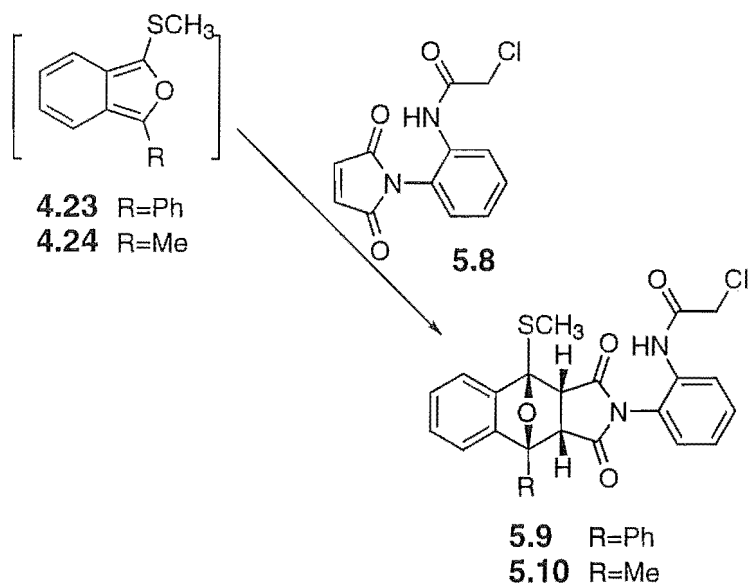


Fig. 5.3.1

The ^1H NMR spectrum of the 3-phenyl-substituted adduct **5.9** contained a characteristic pair of doublets at 4.08ppm and 4.27ppm corresponding to the two bridgehead protons. A two proton singlet at 3.73ppm corresponding to the chloromethyl group showed that the chloroacetamide portion of the dienophile had remained intact in the reaction. This was further confirmed by the mass spectrum which included a peak at 520.1 mass units corresponding to the parent ion as well as a peak at 473.1 mass units corresponding to loss of the chloromethyl group. The pattern of fragmentation around these peaks was consistent with the loss of chlorine, showing the characteristic pattern expected due to the two isotopes ^{35}Cl and ^{37}Cl .

The 3-methyl-substituted adduct **5.10** also gave rise to a characteristic pair of doublets in its ^1H NMR spectrum at 3.10ppm and 3.23ppm corresponding to the bridgehead protons. Once more a two proton singlet at 3.83ppm confirmed the presence of the chloroacetamide substituent, and a singlet resonance due to the 3-methyl group was observed at 2.02ppm. The mass spectrum of **5.10** was analogous to that for the 3-phenyl-substituted **5.9**, containing a parent ion at 442

mass units along with a peak at 395 mass units corresponding to loss of the chloromethyl group.

5.3.2 Aromatisation

The Diels-Alder adducts **5.9** and **5.10** were aromatised by treatment of a dichloromethane solution with trifluoroacetic acid (TFA) as shown in Fig. 5.3.2.

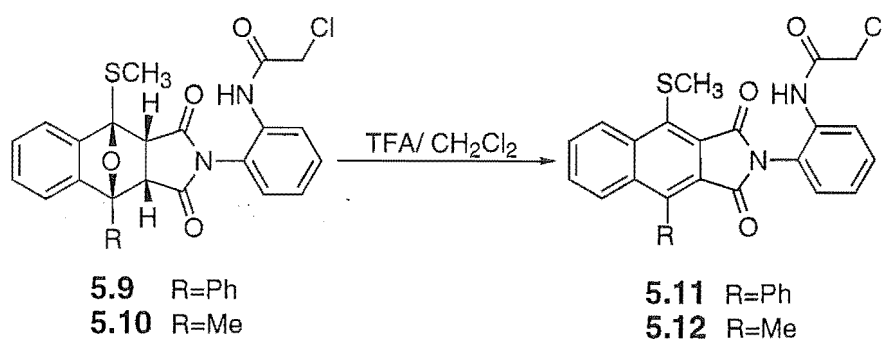


Fig. 5.3.2

This resulted in the direct formation of the desired aromatised derivatives **5.11** and **5.12** respectively, although traces of the intermediate 3-hydroxy substituted compounds were also observed.

The characteristics of these two aromatised products were an absence of the doublet pairs which occurred between 3.0 and 4.5ppm in the ¹H NMR spectra of the bridged adducts, along with shifts in the aryl proton peaks, and mass spectra containing a parent ion along with peaks corresponding to the loss of Cl, CH₃Cl, and COCHCl fragments.

5.3.3 Biological Testing

In vitro P388 anti-tumour biological testing of these compounds (see Appendix) gave interesting results. An effective prodrug requires the potency of the prodrug to be low relative to the potency of the drug itself. The 3-phenyl-substituted adduct **5.9** gave an ID₅₀ value of 1.26 µg/mL. As expected from steric considerations, the 3-methyl-substituted adduct **5.10** gave a lower result, an encouraging 0.42 µg/mL. Contrary to expectation however, the corresponding bridge-opened planar compounds **5.11** and **5.12** displayed higher ID₅₀ values, 3.03 and 2.52 µg/mL respectively. This could indicate that the oxygen-bridged adducts are more easily taken up by the tumour cells, and are perhaps being bridge-opened to their planar forms *in situ*. This possibility led to the synthesis and anti-tumour testing of more stable sulfur-bridged adducts, as described in Chapter 6.

5.3.4 Summary

Several Diels-Alder adducts and their aromatised derivatives were synthesised with the chloroacetamide functionality introduced at the *ortho* position of the *N*-aryl group. These compounds displayed intriguing anti-tumour activity *in vitro*.

5.4 Dienophiles with Hydrogen-Bonding Functionality

We also undertook the synthesis of dienophiles capable of hydrogen-bonding with nucleophilic sites in DNA. This required the introduction of hydrogen-bonding moieties into *N*-aryl maleimide based dienophiles. A convergent

synthetic approach was used, with *N*-(3-carboxyphenyl) maleimide **5.13** chosen as the general starting compound for a series of coupling reactions.

Fig. 5.4.1 shows the synthesis of **5.13** from maleic anhydride and 3-amino benzoic acid, in a preparation analogous to that for *N*-phenyl maleimide. This reaction proceeded smoothly, giving **5.13** in 90% yield.

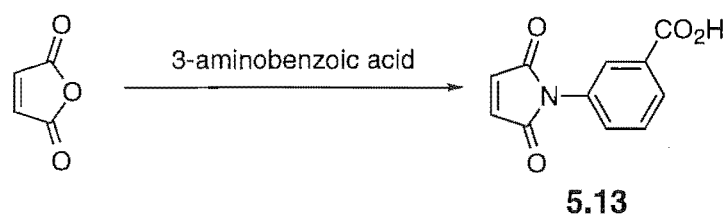


Fig. 5.4.1

The synthesis of amide derivatives of **5.13** was accomplished by direct coupling of an amine and this carboxylic acid. This coupling was brought about by the use of dicyclohexylcarbodiimide (DCC). The proposed general mechanism of this reaction is illustrated in Fig. 5.4.2.⁷⁷

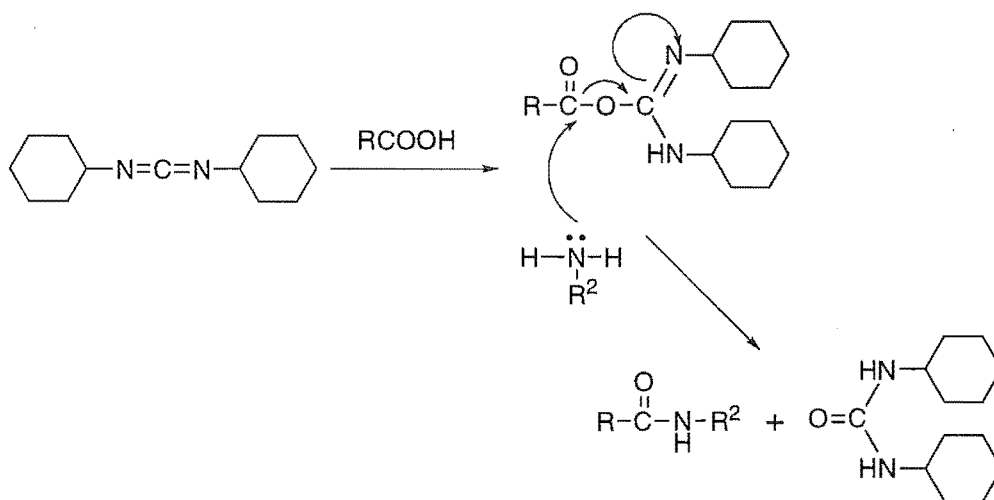


Fig. 5.4.2 Proposed mechanism of DCC coupling reaction

The reaction commences with the acylation of DCC by the carboxylic acid, thereby activating it for nucleophilic attack. The amine, present also in the reaction mixture, attacks as the nucleophile, displacing the DCC and forming a peptide bond in the process. The products are *N,N'*-dicyclohexylurea (DCU) and an amide.

The use of 1-hydroxybenzotriazole (HOBt) as a catalyst in the reaction generally enhances the efficiency and specificity of the coupling by intercepting the acyl urea and forming a highly reactive *O*-acyl-1-hydroxybenzotriazole intermediate.

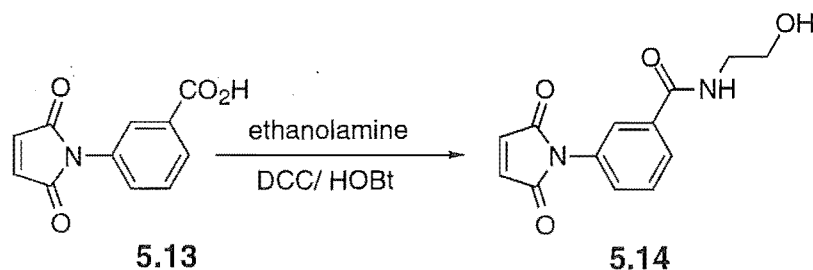


Fig. 5.4.3

The coupling reaction of **5.13** with ethanolamine using DCC and HOBt is outlined in Fig. 5.4.3. This resulted in the formation of the corresponding *N*-aryl maleimide compound **5.14**. The ¹H NMR spectrum of **5.14** was characterised by a singlet at 6.87ppm corresponding to the two maleimide protons, which indicated that the maleimide ring had remained intact in the coupling process. Multiplet resonances at 3.46ppm and 3.63ppm corresponding to the ethanolic moiety indicated that the coupling had been successful.

Compound **5.14** had to be used immediately as a dienophile, as it was found to decompose rapidly. The maleimide ring fragmented upon storage, as indicated in the ¹H NMR spectrum by the appearance of two doublet resonances, in place of the singlet at 6.87ppm.

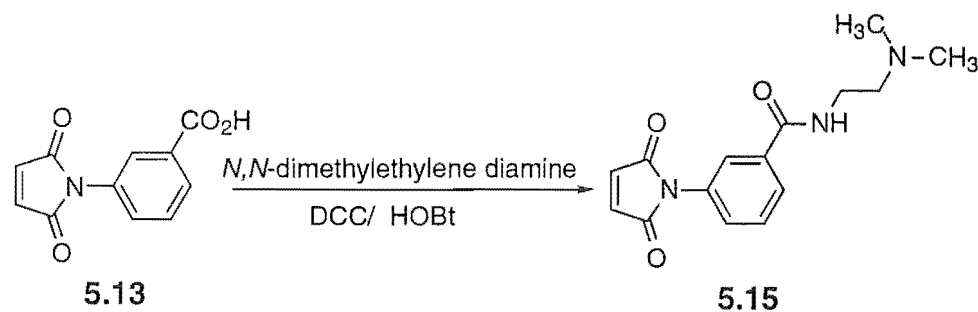


Fig. 5.4.4

The next coupling reaction involved the introduction of a dimethylamino-substituted side chain. This is shown in Fig. 5.4.4. In this reaction the carboxylic acid **5.13** was coupled to *N,N*-dimethylethylenediamine to give as the product *N*-(3-(2-aza-4-dimethylamino-1-oxobutanyl)phenyl) maleimide **5.15**. This *N*-aryl maleimide derivative was characterised in the ¹H NMR spectrum by a singlet at 6.84ppm corresponding to the two maleimide protons. Once more, relatively rapid decomposition involving maleimide ring opening was observed on storage, and **5.15** was used immediately in Diels-Alder chemistry.

In analogy of systems with strong DNA affinity synthesised by Dervan *et al.*, this system was further extended by the addition of an extra linking section, as shown in Fig. 5.4.5.⁶¹ 3-Aminobenzoic acid **5.16** was first coupled with *N,N*-dimethylethylenediamine to give the amine **5.17**. In turn this was coupled to *N*-(3-carboxyphenyl) maleimide **5.13** to give the *N*-aryl maleimide **5.18**, which contained a characteristic maleimide singlet at 6.85ppm in its ¹H NMR spectrum. However this *N*-aryl maleimide was found to decompose very rapidly, and as a consequence, it was not used in Diels-Alder chemistry.

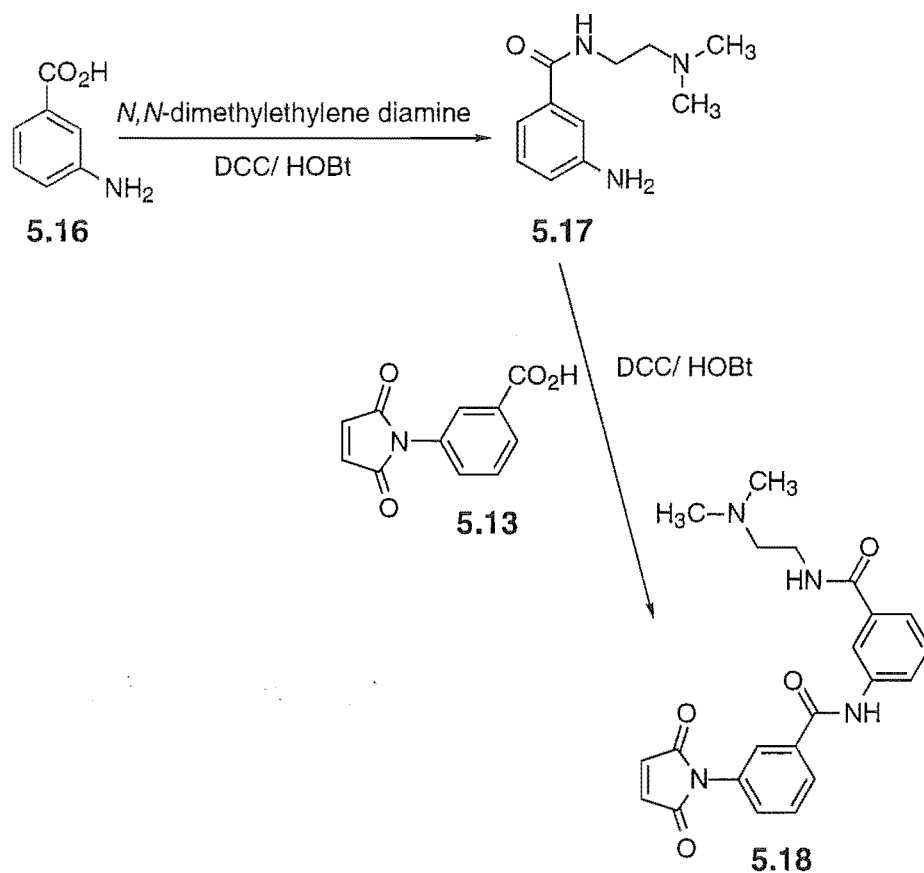


Fig. 5.4.5

Once more with the aim of enhancing hydrogen-bonding interactions with DNA, a pyridyl moiety was introduced into the system. Fig. 5.4.6 outlines this synthesis.

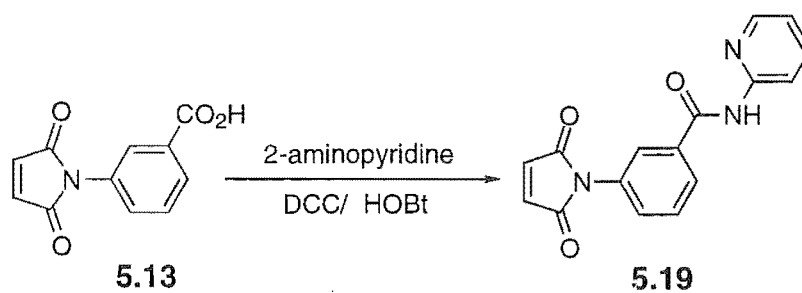


Fig. 5.4.6

N-(3-Carboxyphenyl) maleimide **5.13** was coupled to 2-aminopyridine to give the *N*-aryl maleimide **5.19**, with a characteristic singlet corresponding to its two maleimide protons at 6.93ppm in the ^1H NMR spectrum. As with the related compounds already mentioned, **5.19** decomposed on storage and was used immediately as a dienophile in subsequent Diels-Alder chemistry.

5.5 Diels-Alder Chemistry of Functionalised Dienophiles

This section describes the Diels-Alder chemistry of functionalised dienophiles detailed in Section 5.4, along with the bridge cleavage and aromatisation of the resulting adducts.

5.5.1 Diels-Alder Reactions

The dienes 1-methylsulfanyl-3-phenyl-isobenzofuran **4.23** and 1-methylsulfanyl-3-methyl-isobenzofuran **4.24** were prepared *in situ* and reacted with the freshly synthesised dienophile **5.14** as shown in Fig. 5.5.1.

These Diels-Alder reactions gave the desired adducts **5.20** and **5.21** respectively. The ^1H NMR spectrum of the 3-phenyl-substituted adduct **5.20** included characteristic bridgehead proton doublet resonances at 4.01 and 4.27ppm, while those for the 3-methyl-substituted adduct **5.21** were at 3.08 and 3.21ppm.

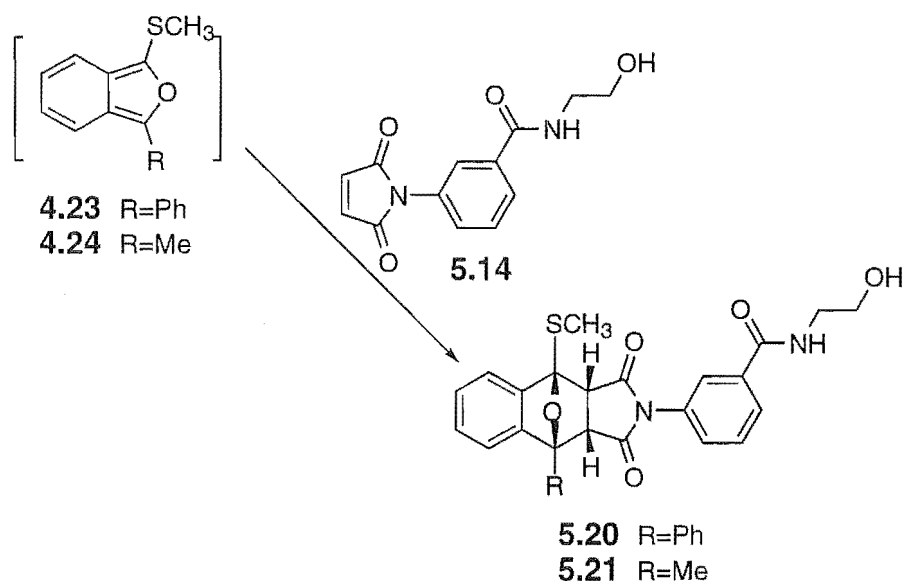


Fig. 5.5.1

The dienes 1-methylsulfanyl-3-phenyl-isobenzofuran **4.23** and 1-methylsulfanyl-3-methyl-isobenzofuran **4.24** were also reacted in Diels-Alder fashion with the freshly synthesised dienophile **5.15**, as shown in Fig. 5.5.2. In these instances the desired adducts, **5.22** or **5.23** respectively, could not be isolated.

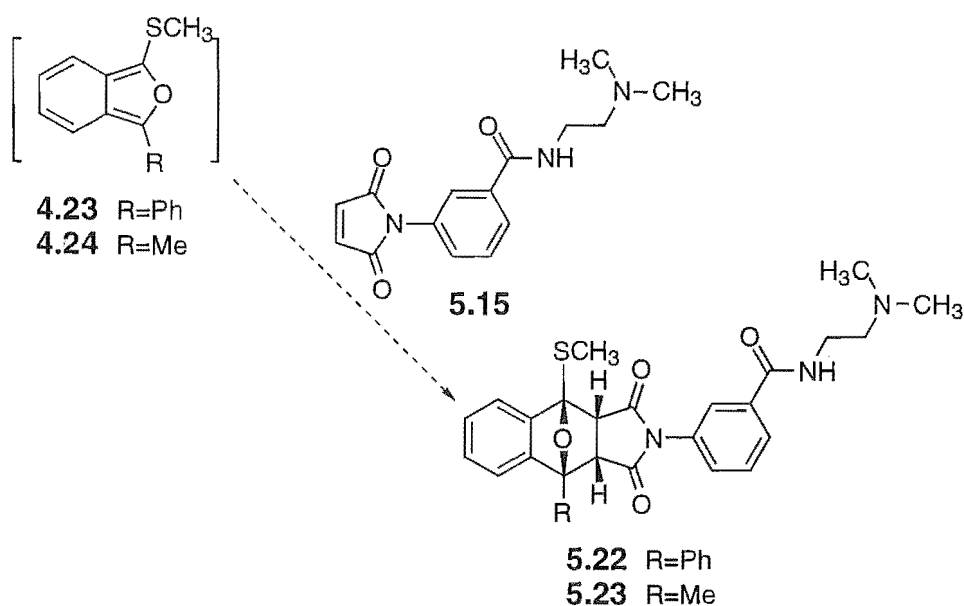


Fig. 5.5.2

In like manner 1-methylsulfanyl-3-phenyl-isobenzofuran **4.23** and 1-methylsulfanyl-3-methyl-isobenzofuran **4.24** were reacted in Diels-Alder fashion with the dienophile **5.19**, as shown in Fig. 5.5.3.

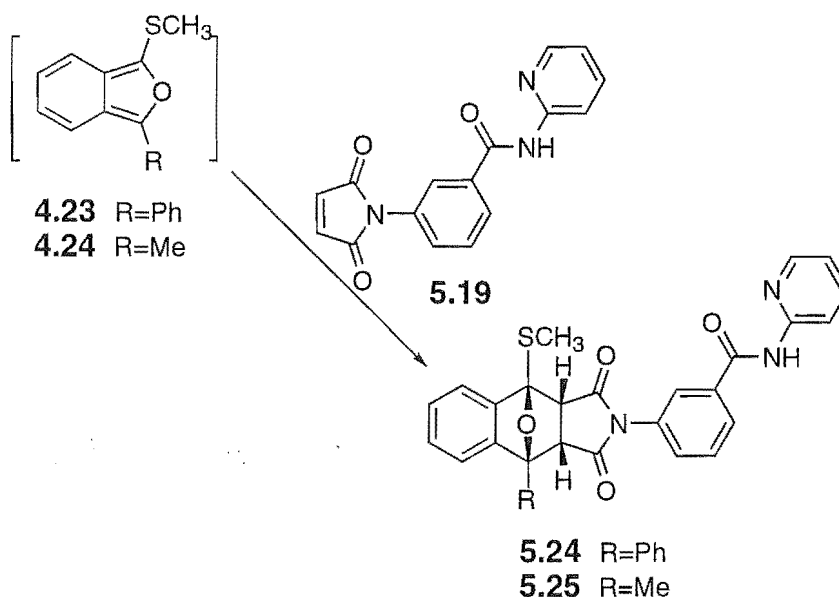


Fig. 5.5.3

These reactions were both stereospecific, leading to the formation of the desired adducts **5.24** and **5.25** respectively. Once again the presence of two bridgehead proton doublet resonances at around 4ppm in the ¹H spectra were indicative of Diels-Alder adduct formation. The mass spectra of these two adducts were characterised by the presence of only a very small parent ion peak but of a stronger peak corresponding to the loss of a C₅H₂N fragment.

5.5.2 Bridge Cleavage

The bridge cleavage and aromatisation of the Diels-Alder adducts **5.20** and **5.21** is shown in Fig. 5.5.4. The adducts were dissolved in dichloromethane and

trifluoroacetic acid added, resulting in cleavage of the bridge and formation of the aromatised products **5.27** and **5.28** respectively.

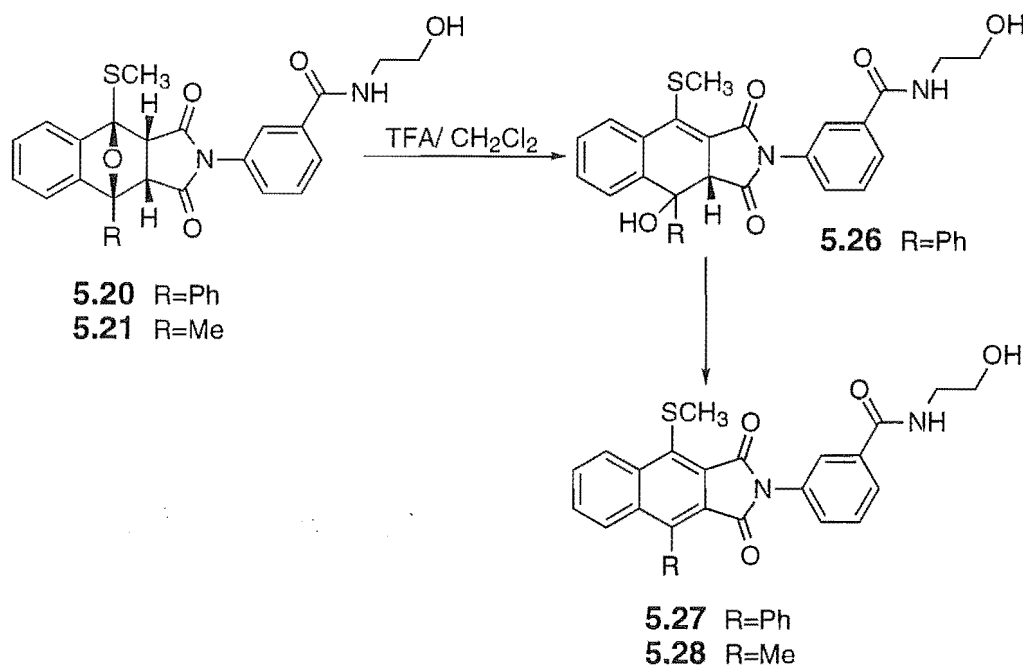


Fig. 5.5.4

In the 3-phenyl case, the intermediate 3-hydroxy compound **5.26** was also observed, as evidenced by a singlet resonance at 4.10ppm in the ¹H NMR spectrum. Further stirring with trifluoroacetic acid resulted in the disappearance of this singlet, indicating that the 3-hydroxy compound had all been dehydrated to the aromatic derivative **5.27**. Relative to their oxygen-bridged precursors, the ¹H NMR spectra of **5.27** and **5.28** were characterised by a downfield shift in the aromatic protons along with disappearance of the bridgehead proton doublet resonances.

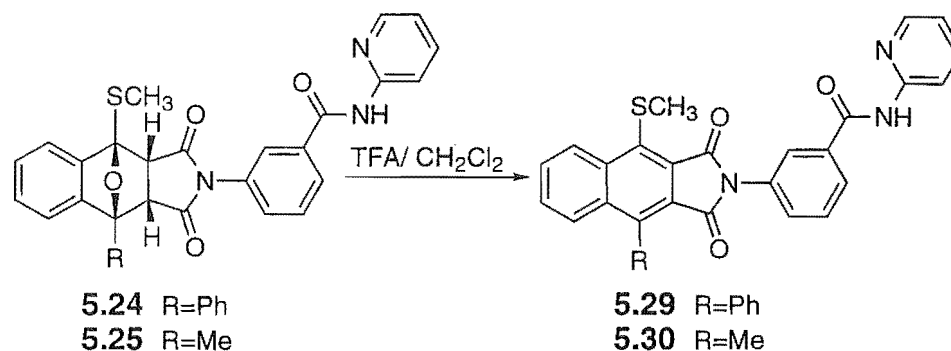


Fig. 5.5.5

Similarly the Diels-Alder adducts **5.24** and **5.25** were aromatised as shown in Fig. 5.5.5 to give the expected products **5.29** and **5.30** respectively. The mass spectrum of the 3-phenyl-substituted compound **5.29** contained a weak parent ion peak and two stronger peaks corresponding to the loss of C₅H₂N and C₅H₅N₂ fragments. The mass spectrum of the 3-methyl-substituted compound **5.30** on the other hand, contained a weak parent ion along with peaks corresponding to loss of C₂H₆S and C₅H₃N fragments. Once again, relative to their oxygen-bridged precursors, the ¹H NMR spectra of **5.29** and **5.30** showed a downfield shift in the aromatic protons and disappearance of the bridgehead proton doublets.

5.5.3 Biological Testing

The hydroxy-substituted adducts, **5.20** and **5.21**, displayed moderate activity in the P388 biological assay, giving ID₅₀ values of 2.27 and 8.15 μg/mL respectively. However as with the alkylating products synthesised previously, the bridge-opened planar aromatic compounds **5.27** and **5.28** displayed less activity than their relative precursors, giving ID₅₀ values of 34.60 and 51.00 μg/mL respectively. The oxygen-bridged pyridine-substituted compounds, **5.24** and **5.25**, both displayed moderate cytotoxic activity, giving ID₅₀ values of 10.99 and 4.99 μg/mL

respectively. However once again, the bridge-opened planar aromatic compounds **5.29** and **5.30** displayed less activity than their relative precursors, giving ID₅₀ values of >6.25 and 8.1 $\mu\text{g/mL}$ respectively.

5.6 Conclusion

A range of oxygen-bridged Diels-Alder adducts containing functional groups of interest were successfully synthesised. These included the synthesis of adducts with alkylating substituents, and adducts with groups capable of hydrogen-bonding interactions with nucleophiles. These adducts were also successfully bridge-opened to give planar aromatic products with potential intercalating ability. Biological testing of compounds synthesised in this chapter gave intriguing results. Both those compounds containing a hydrogen-bonding functionality in the side chain and those containing an alkylating functionality showed significant activity. In these series, however, the bridged precursors were found to be more cytotoxic than the corresponding aromatic derivatives. This result directed attention to the preparation of Diels-Alder adducts with a more stable bridge. To this end, Chapter 6 deals with the synthesis of sulfur-bridged Diels-Alder adducts.

Chapter 6

SULFUR-BRIDGED DIELS- ALDER ADDUCTS

6.1 Introduction

Stability is an important factor in the Diels-Alder chemistry of isobenzofurans. If an isobenzofuran is too unstable then it may decompose before it can be trapped by a dienophile. On the other hand if it is very stable it can be isolated, but may be less reactive as a diene.²³ Another factor under consideration in this thesis is the stability of the bridge of Diels-Alder adducts resulting from reaction with dienophiles. This bridge must be stable enough to prevent spontaneous cleavage under biological conditions, which would lead to toxicity problems. If too stable however, then cleavage itself, and thereby activation of the drug, could present difficulties.

The investigation of isobenzothiophene Diels-Alder chemistry presented in this chapter was undertaken in response to the search for an appropriate balance. Isobenzothiophenes are inherently more stable than the corresponding isobenzofurans. Sulfur-bridged Diels-Alder adducts, too, offer more stability than their oxygen-bridged counterparts. This chapter describes the evaluation of the

synthesis of such sulfur-bridged Diels-Alder adducts and their subsequent bridge cleavage.

6.2 Isobenzothiophenethiones

In order to explore the Diels-Alder chemistry of isobenzothiophenes it was first necessary to synthesise appropriate precursors. This section describes the synthesis of these precursors, isobenzothiophenethiones, which were used to generate the corresponding isobenzothiophenes.

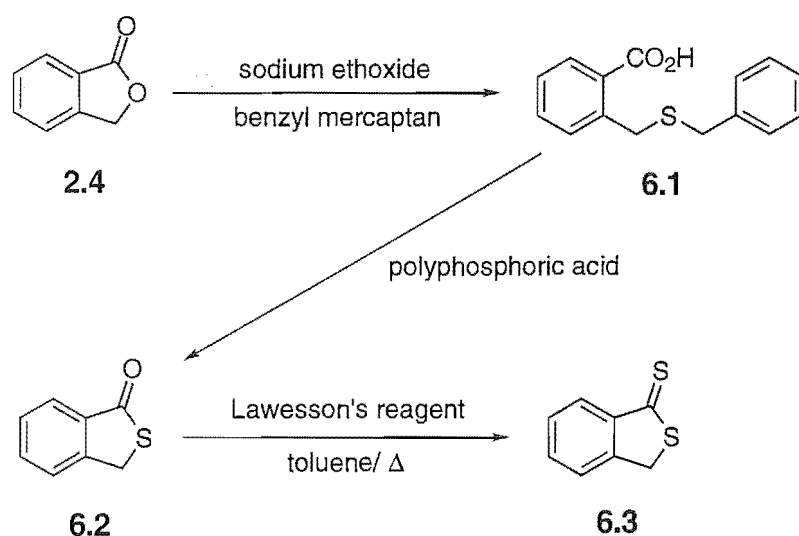


Fig. 6.2.1

Fig. 6.2.1 outlines the synthesis of 1(3H)-isobenzothiophene-1-thione **6.3**. 2-(Benzylmercaptomethyl)benzoic acid **6.1** was generated by the nucleophilic attack of benzyl mercaptan on 1(3H)-isobenzofuranone **2.4**, and then cyclised to 1(3H)-isobenzothiophenone **6.2** using polyphosphoric acid.⁷⁸ The generation of **6.2** from the starting material **2.4** was clearly evidenced by a change in the chemical shift of the benzylic protons from 5.35ppm in **2.4** to 4.48ppm in **6.2**. Thionation using Lawesson's reagent was then performed in a manner analogous

to that used to generate isobenzofuranthiones, resulting in the formation of 1(3H)-isobenzothiophenethione **6.3** as desired. This was characterised by ^1H NMR spectroscopy, with a particularly distinctive singlet resonance observed at 4.53ppm.⁷⁹

The synthesis of 3-phenyl-1(3H)-isobenzothiophene-1-thione **6.5** was accomplished in a single step as outlined in Fig. 6.2.2. 2-Benzoylbenzoic acid **6.4** was thionated with Lawesson's reagent to give 3-phenyl-1(3H)-isobenzothiophene-1-thione **6.5** directly.⁷⁹

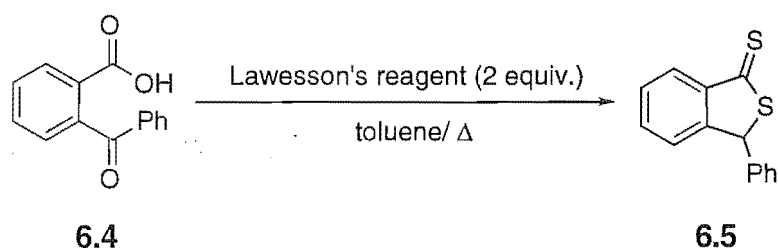


Fig. 6.2.2

The synthesis of 3-methyl-1(3H)-isobenzothiophene-1-thione was carried out in an analogous fashion to that of the phenyl-substituted case. This is outlined in Fig. 6.2.3.

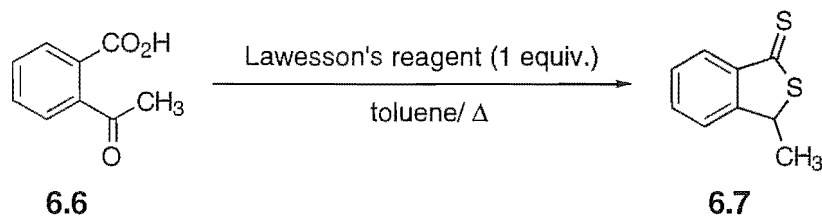


Fig. 6.2.3

Treatment of 2-acetylbenzoic acid **6.6** with one equivalent of Lawesson's reagent under reflux in toluene gave the desired product 3-methyl-1(3H)-isobenzothiophene-1-thione **6.7**.⁷⁹ The ^1H NMR spectrum included a doublet

resonance at 1.78ppm, and a quartet at 4.93ppm corresponding to the methyl group and the benzylic hydrogen respectively.

The isobenzothiophenethiones **6.3**, **6.5**, and **6.7** were used to generate dienes for appropriate Diels-Alder chemistry.

6.3 Sulfur-Bridged Diels-Alder Adducts

This section describes an investigation of the Diels-Alder chemistry of isobenzothiophenes with simple dienophiles, and the bridge cleavage of the resulting adducts. Section 6.4 discusses the extension of this Diels-Alder chemistry to functionalised dienophiles.

6.3.1 Diels-Alder Chemistry

The synthesis of sulfur-bridged Diels-Alder adducts was initiated by evaluating the cycloaddition of 1-methylsulfanyl-isobenzothiophene **6.8** with dimethyl fumarate, as shown in Fig. 6.3.1. The synthesis of the diene was carried out in an analogous fashion to the isobenzofuran syntheses developed earlier.

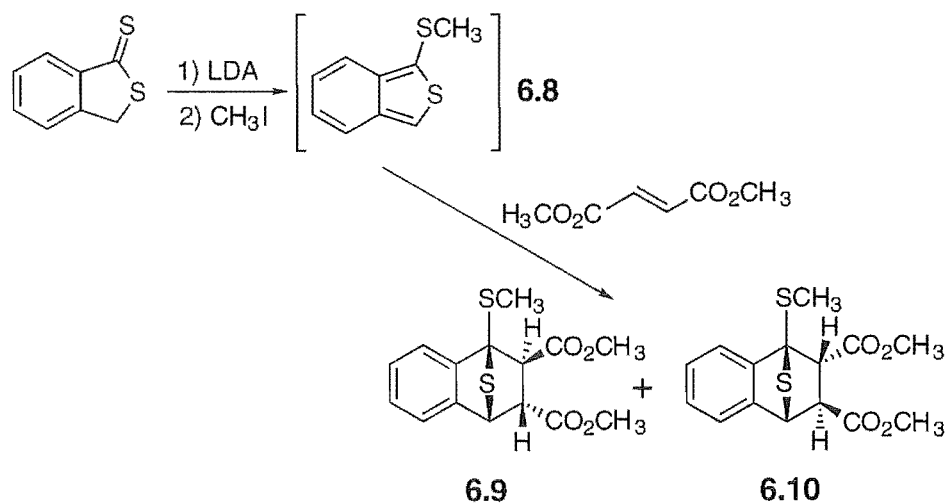


Fig. 6.3.1

1(3H)-Isobenzothiophenethione **6.3** was converted to 1-methylsulfanyl-isobenzothiophene **6.8** *in situ* using LDA and methyl iodide. This was then trapped with dimethyl fumarate, to give a mixture of two isomeric adducts, **6.9** and **6.10**, in 9% yield. The mass spectrum of this mixture was characterised by a peak corresponding to the parent ion at 324 mass units, along with a peak at 180 mass units due to retro-Diels-Alder fragmentation.

In the case of 1-methylsulfanyl-isobenzofuran, trapping with *N*-phenyl maleimide was only effective when substituents were present at the 3-position of the diene. Such substitution restricts the opportunities for prodrug design and activation. We evaluated whether the enhanced stability of 1-methylsulfanyl-isobenzothiophene, relative to 1-methylsulfanyl-isobenzofuran, would result in a successful Diels-Alder reaction with *N*-phenyl maleimide. This reaction is outlined in Fig. 6.3.2.

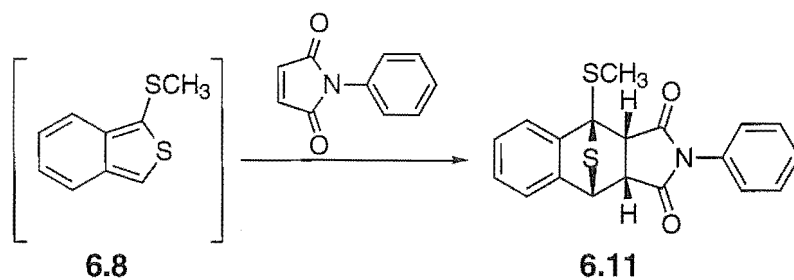


Fig. 6.3.2

The reaction resulted in the isolation of a single Diels-Alder adduct **6.11**, in 6% yield. This was identified as the *endo* isomer by its ¹H NMR spectrum, which contained a characteristic pair of doublet resonances at 3.25 and 3.57ppm. In adducts such as this, with no substitution at the 3-position of the diene, the *exo* isomer is characterised by a singlet resonance and a multiplet resonance corresponding to the bridgehead hydrogens, rather than two doublet resonances.

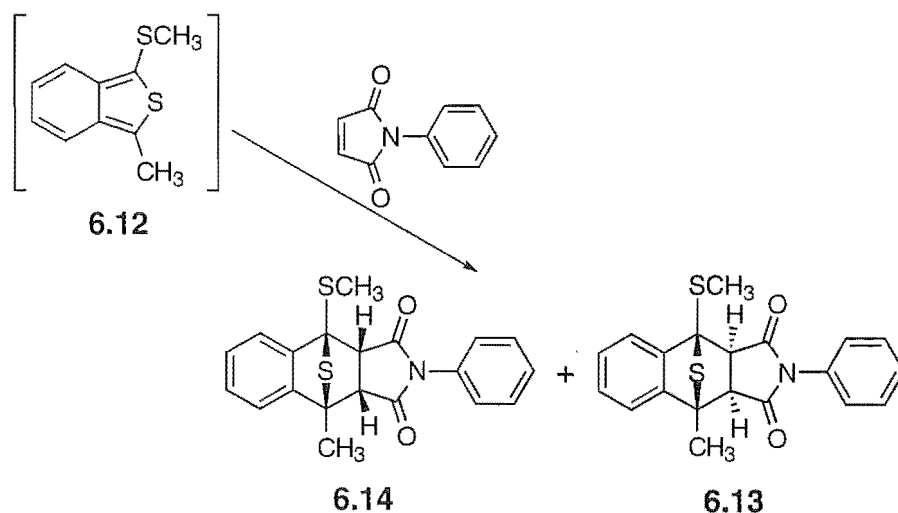


Fig. 6.3.3

The 3-methyl substituted isobenzothiophene **6.12** was likewise generated *in situ* and trapped with *N*-phenyl maleimide as shown in Fig. 6.3.3. In this case a pair of isomeric adducts, **6.13** and **6.14**, was isolated, in 7% yield and in a 5 to 1 ratio. The *endo* isomer **6.14** was identified as the major isomer by comparison with analogous adducts. The ¹H NMR spectrum included two pairs of doublet resonances corresponding to the bridgehead protons, at 4.07 and 4.23ppm for **6.14**, and 3.21 and 3.34ppm for **6.13**.

Section 6.3b describes the bridge cleavage of these sulfur-bridged Diels-Alder adducts.

6.3.2 Bridge Cleavage

The enhanced stability of the sulfur bridge requires a reagent other than TFA for bridge cleavage in the model system. The method chosen for bridge cleavage of these Diels-Alder adducts was mercuric acetate catalysed hydrolysis. The proposed mechanism for such a cleavage is outlined in Fig. 6.3.4.

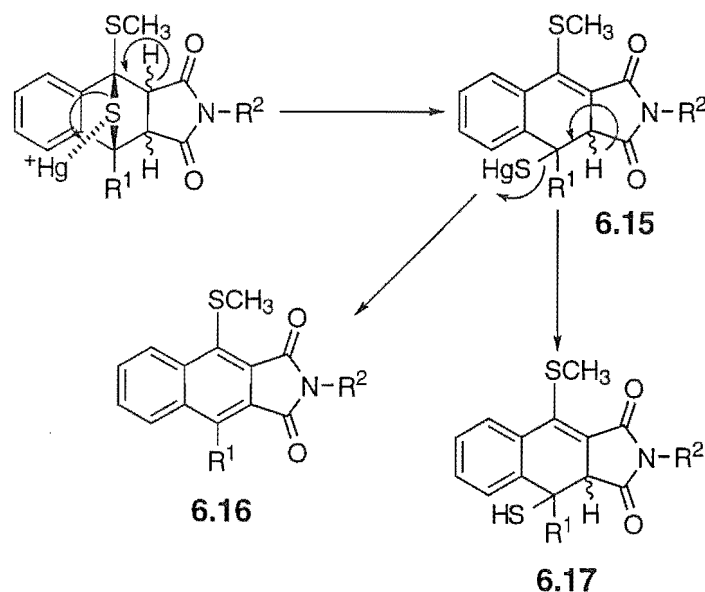


Fig. 6.3.4 Proposed mechanism of bridge cleavage

The large positively charged mercury ion can associate with the sulfur bridge, catalysing bridge cleavage to give **6.15**. This may be followed by the loss of another hydrogen as shown to give the planar aromatic **6.16**. Alternatively, the mercury cation may dissociate from the sulfur atom of **6.15**, leading to the non-planar product **6.17**.

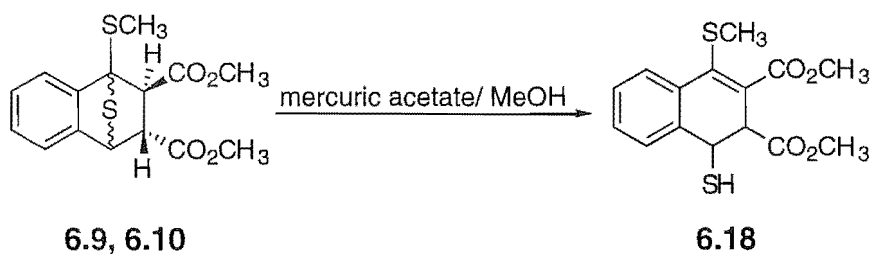


Fig. 6.3.5

Fig. 6.3.5 outlines the bridge cleavage of the isomeric mixture of **6.9** and **6.10** with mercuric acetate, to give a dihydronaphthalene derivative **6.18**. Both the ¹H NMR spectrum and mass spectrum of **6.18** were analogous to those of the

hydroxy-substituted product synthesised in previous work on oxygen-bridged Diels-Alder adducts.

The *N*-phenyl maleimide derived Diels-Alder adduct **6.11** underwent an analogous elimination reaction on treatment with mercuric acetate, as shown in Fig. 6.3.6.

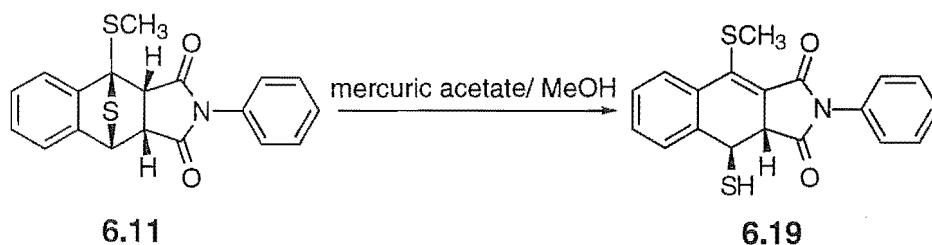


Fig. 6.3.6

The product was found to be **6.19**, as evidenced in the ¹H NMR spectrum by loss of the two bridgehead hydrogen doublet resonances, and a strong shift downfield in the resonances of the aromatic protons relative to those of **6.11**. A new singlet resonance was observed at 3.90ppm corresponding to the remaining bridgehead proton. Mass spectroscopy, using both electron impact (EI) and fast atom bombardment (FAB) techniques, showed a peak corresponding to the parent ion at 353 mass units, as well as a peak at 317 mass units, corresponding to its aromatic derivative, formed by loss of SH₂.

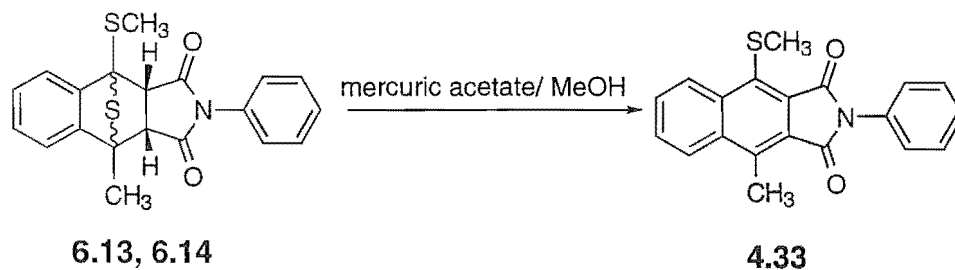


Fig. 6.3.7

A different result was observed in the case of the analogous 3-methyl substituted Diels-Alder adducts **6.13** and **6.14**, as shown in Fig. 6.3.7. In this case the product was identified by ^1H NMR as the aromatic derivative **4.33**, which had been both synthesised and characterised in previous work on oxygen-bridged Diels-Alder adducts. This result, along with those of subsequent sulfur-bridge cleavage reactions, indicates that when alkyl substituents are present at the 3-position, bridge cleavage by mercuric acetate catalysed hydrolysis leads to aromatisation. This parallels the situation observed in the oxygen-bridged adducts.

6.3.3 Summary

These Diels-Alder reactions of 1-methylsulfanyl-isobenzothiophene and 1-methylsulfanyl-3-methyl-isobenzothiophene demonstrated the feasibility of synthesising sulfur-bridged compounds in line with our model systems. Such isobenzothiophenes displayed more stability than their isobenzofuran counterparts, resulting in increased yields from Diels-Alder reactions. The sulfur bridge present in the Diels-Alder adducts themselves was also found to be more stable than analogous oxygen-bridged adducts. Two pathways were observed to be followed for bridge cleavage by mercuric acetate, with alkyl substitution at the 3-position resulting in aromatisation.

6.4 Functionalised Diels-Alder Adducts

With the feasibility of synthesising sulfur-bridged Diels-Alder adducts demonstrated, we now wished to investigate the introduction of functional groups capable of interacting with DNA. This section describes the synthesis of such

adducts via functionalised dienophiles and the subsequent bridge cleavage of the resulting adducts.

6.4.1 Introduction of Hydrogen-Bonding Functional Groups

Electrophilic moieties attached to an intercalator are capable of hydrogen-bonding interactions with nucleophilic sites in DNA. This section explores the introduction of such functionalities into sulfur-bridged Diels-Alder adducts via the dienophile.

In the case of isobenzofurans, Diels-Alder chemistry had proceeded most smoothly with phenyl substitution at the 3 position, likely due to enhanced diene stability. It was hoped that use of the corresponding 3-phenyl substituted isobenzothiophene would, likewise, result in successful Diels-Alder reactions with functionalised dienophiles.

Rather than prepare this diene *in situ*, 1-methylsulfanyl-3-phenyl-isobenzothiophene **6.20** was sufficiently stable to be isolated and purified prior to investigation of its Diels-Alder chemistry.⁸⁰ 3-Phenyl-1(3H)-isobenzothiophene-1-thione **6.5** was converted to **6.20** as shown in Fig. 6.4.1.

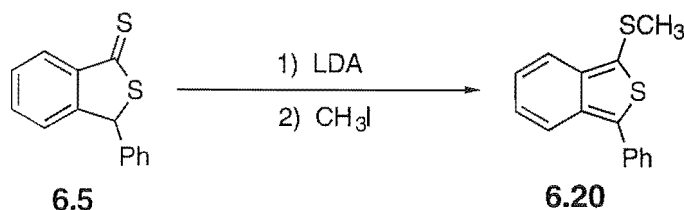


Fig. 6.4.1

1-Methylsulfanyl-3-phenyl-isobenzothiophene **6.20** was reacted with the hydroxy-substituted dienophile **5.16**, as outlined in Fig. 6.4.2.

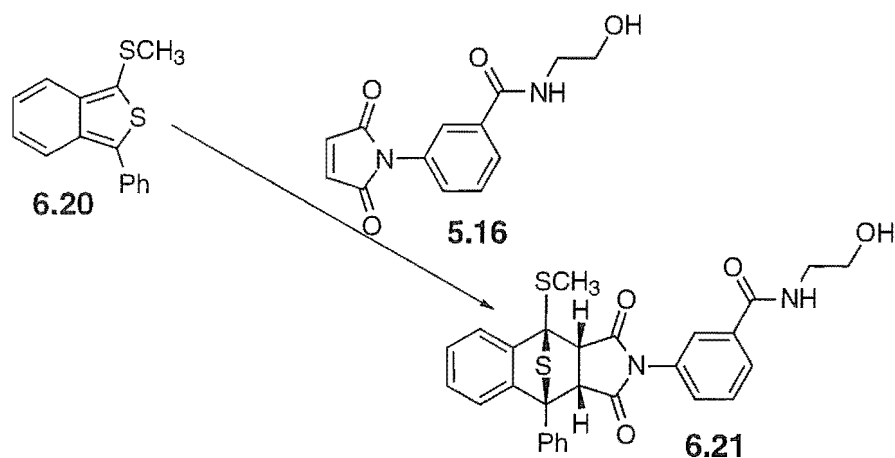


Fig. 6.4.2

This resulted in formation of the desired adduct **6.21**. The ^1H NMR spectrum of **6.21** included resonances corresponding to the bridgehead protons at 4.45 and 4.98ppm, downfield relative to the corresponding oxygen-bridged case, which had corresponding doublets at 4.01 and 4.27ppm.

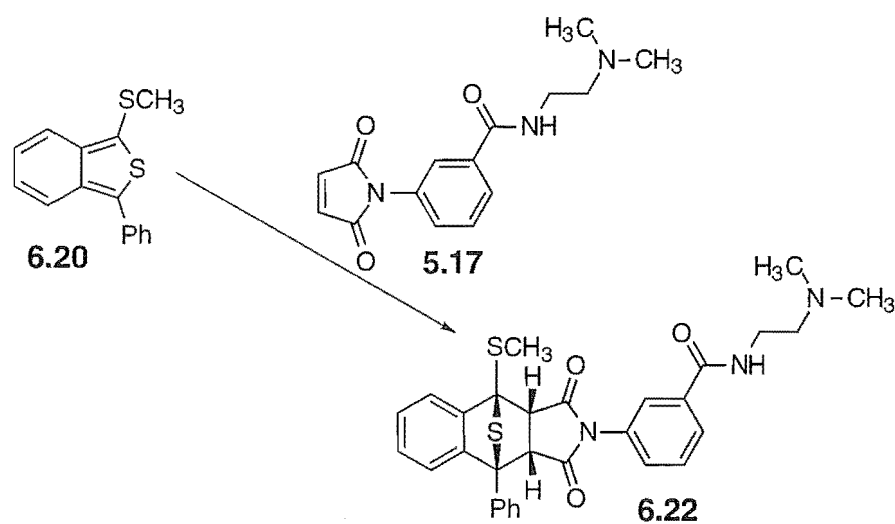


Fig. 6.4.3

Although a less stable dienophile, **5.17** also reacted successfully with 1-methylsulfanyl-3-phenyl-isobenzothiophene **6.20**. The reaction, to give the adduct **6.22**, is shown in Fig. 6.4.3. The yield of this reaction was low (4%),

however it compared favourably with the oxygen-bridged case, where none of the desired adduct was isolated in the analogous reaction.

The Diels-Alder adduct **6.22** itself proved relatively unstable, decomposing upon prolonged freezer storage. The mass spectrum of **6.22** exhibited features corresponding to retro-Diels-Alder fragmentation, highly characteristic of these Diels-Alder adducts. The parent ion peak itself, at 543 mass units, was fairly weak, but sufficiently intense to allow high resolution data to be obtained.

1-Methylsulfanyl-3-phenyl-isobenzothiophene **6.20** was also reacted with the pyridinyl-substituted dienophile **5.21**, another relatively unstable dienophile, as outlined in Fig. 6.4.4.

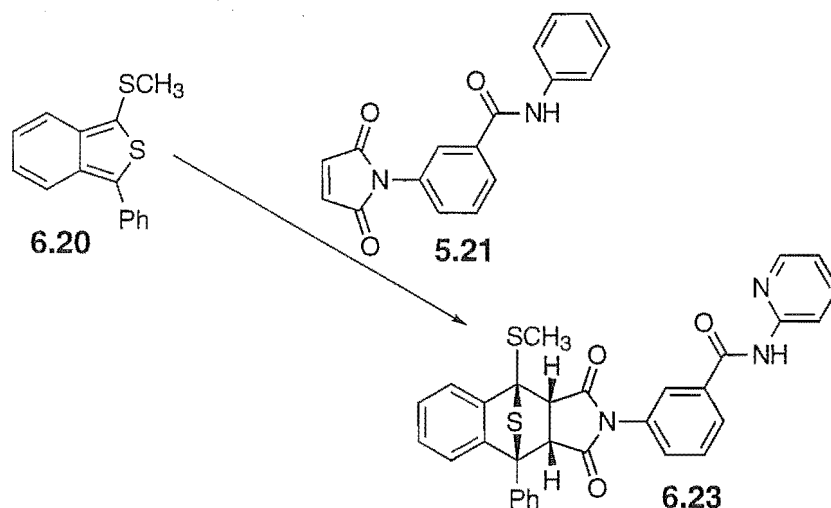


Fig. 6.4.4

This resulted successfully in the formation of the desired Diels-Alder adduct **6.23**, once more in 4% yield. As with the other functionalised sulfur-bridged adducts, only the *endo* isomer was isolated, as identified by comparison with analogous adducts. Once again, the resonances in the ¹H NMR spectrum corresponding to the bridgehead protons were shifted downfield relative to the

corresponding oxygen-bridged case **5.24**, from 3.96 and 4.20ppm in **5.24** to 4.35 and 4.98ppm in the case of **6.23**.

6.4.2 Introduction of an Alkylating Group

As discussed in Section 5.2, alkylating agents are an important class of anti-tumour agent. This section explores the introduction of an alkylating group into sulfur-bridged Diels-Alder adducts.

An attempt was made to trap the diene 1-methylsulfanyl-isobenzothiophene **6.8** with *N*-(2-chloroacetamidophenyl)maleimide **5.10** as outlined in Fig. 6.4.5. However, none of the desired product **6.24** was isolated.

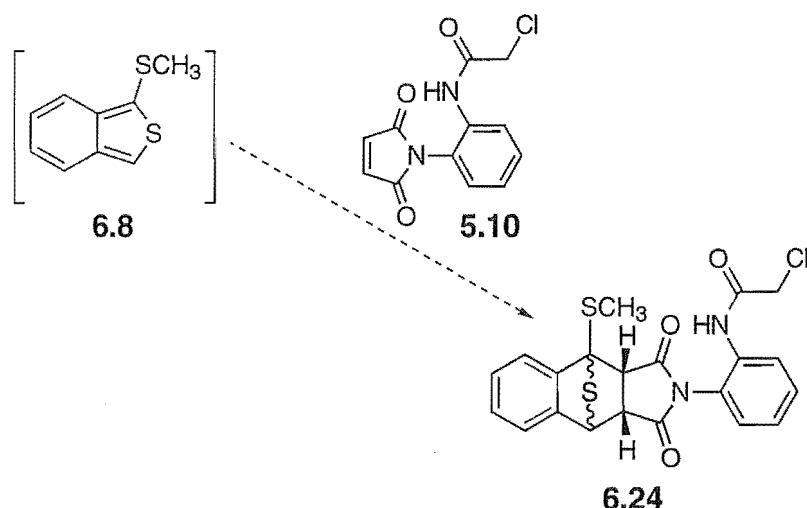


Fig. 6.4.5

A more encouraging result was observed with aryl substitution at the 3-position of the diene. 1-Methylsulfanyl-3-phenyl-isobenzothiophene **6.20** was reacted with *N*-(2-chloroacetamidophenyl)maleimide **5.10** as outlined in Fig. 6.4.6.

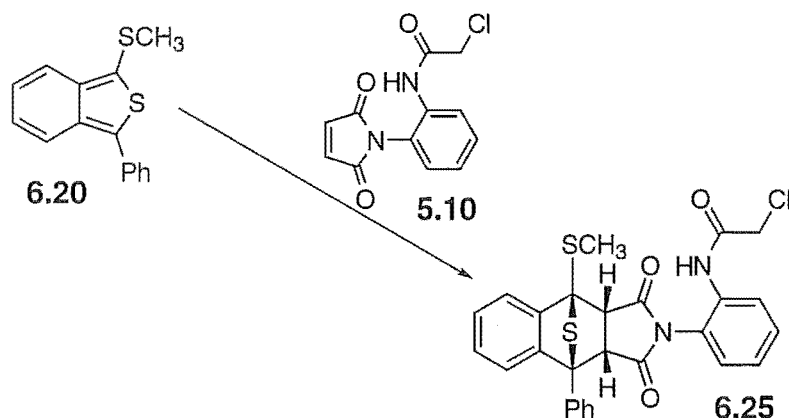
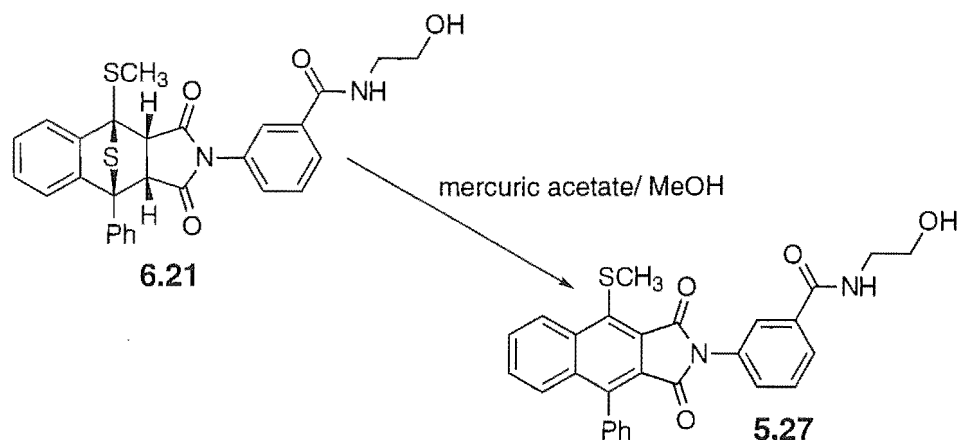


Fig. 6.4.6

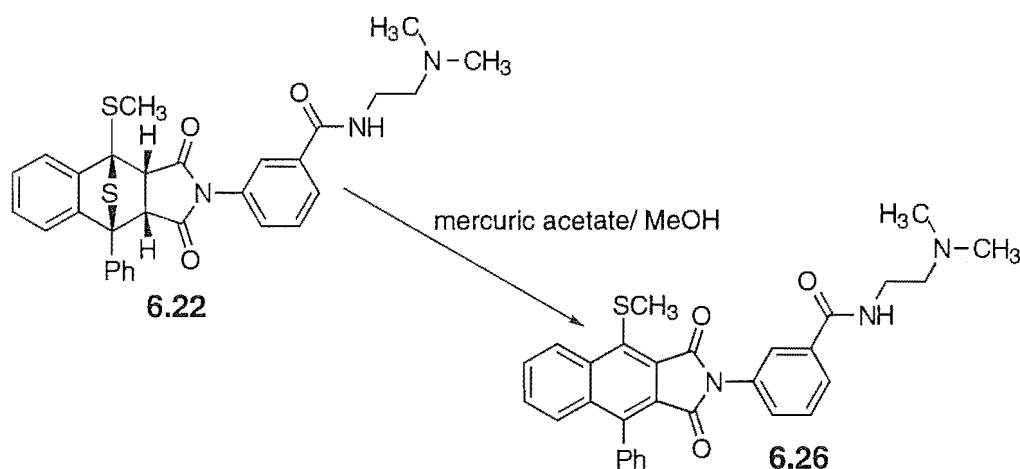
This reaction proceeded smoothly to form the desired sulfur-bridged adduct **6.25**. Only one stereoisomer, the *endo* product, was observed. The ¹H NMR spectrum of **6.25** was very similar to its oxygen-bridged analogue **5.9**, but with many of the resonances shifted downfield. For example, resonances due to the bridgehead protons were at 4.04 and 4.27ppm in the oxygen-bridged case **5.9**, and 4.45 and 4.96ppm in the sulfur-bridged case **6.25**.

6.4.3 Bridge Cleavage

This section describes the bridge cleavage and aromatisation, by mercuric acetate catalysed hydrolysis, of the functionalised sulfur-bridged adducts which have been described thus far in this chapter.

**Fig. 6.4.7**

The bridge cleavage and aromatisation of the hydroxy-substituted adduct **6.21** by mercuric acetate treatment is shown in Fig. 6.4.7. The desired aromatic compound **5.27**, synthesised previously from the analogous oxygen-bridged adduct **5.20**, was observed as the product.

**Fig. 6.4.8**

The sulfur-bridged adduct **6.22** was only available in small quantities. This made the study of its aromatisation a challenge. Nevertheless it was successfully achieved as shown in Fig. 6.4.8. In this case the aromatic product **6.26** had not been prepared previously. The ¹H NMR spectrum of **6.26** was characterised by

the absence of the doublet resonances, at 4.36 and 4.88ppm, associated with the two bridgehead protons of its precursor **6.22**, along with a relative shift downfield in the aromatic proton peaks, characteristic of bridge cleavage. No parent ion was observed in the mass spectrum, but the peaks observed corresponded to the expected fragmentation products of **6.26**, including a peak at 479 mass units corresponding to loss of the *N*-methyl groups.

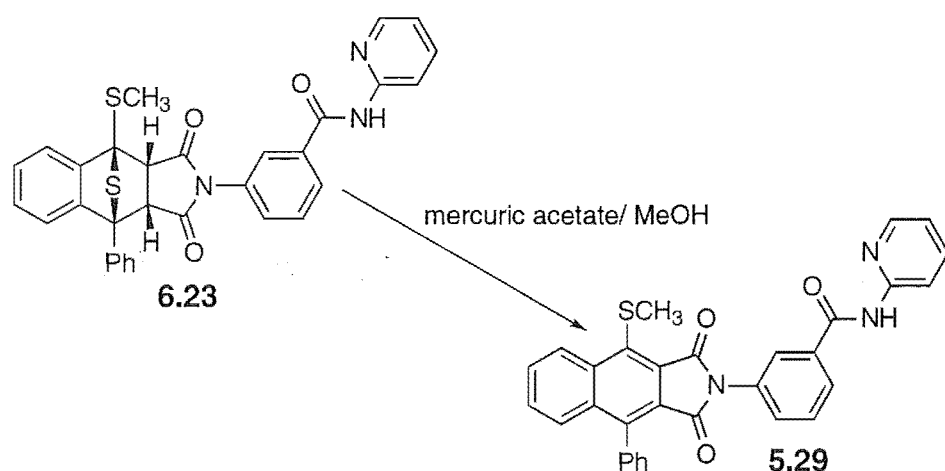


Fig. 6.4.9

The pyridinyl-substituted adduct **6.23** was likewise reacted with mercuric acetate, as outlined in Fig. 6.4.9, to give the desired aromatic compound **5.29**, as synthesised previously from the analogous oxygen-bridged case **5.24**.

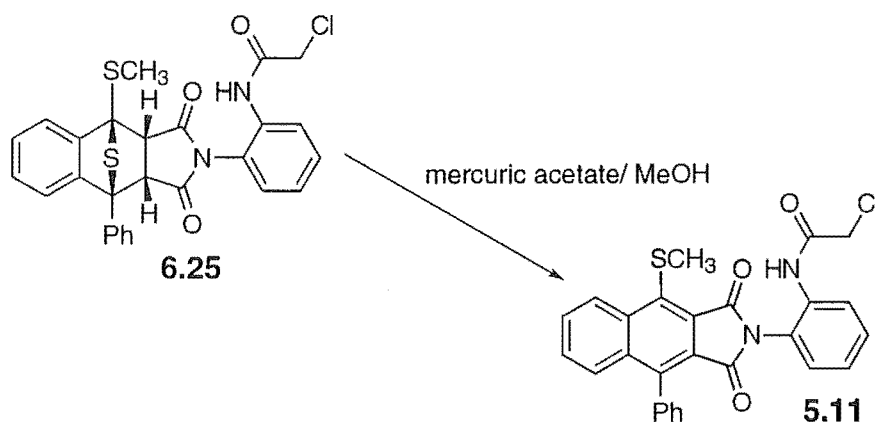


Fig. 6.4.10

The bridge cleavage and aromatisation of **6.25** is shown in Fig. 6.4.10, successfully resulting in the synthesis of **5.11**, an identical product to that formed previously from aromatisation of the corresponding oxygen-bridged case **5.9**.

Thus, all the sulfur-bridged Diels-Alder adducts were able to be successfully bridge cleaved and aromatised by treatment with mercuric acetate.

6.4.4 Biological Testing

In the P388 biological activity assay, the hydroxy-substituted Diels-Alder adduct **6.21** gave an ID₅₀ value of 0.24 µg/mL, surprisingly active compared to its aromatised derivative **5.27**, which displayed little anti-tumour activity (34.60 µg/mL). The adduct **6.22** was more promising, giving an ID₅₀ value of 7.98 µg/mL, while its aromatised derivative **6.26** gave a value of 2.86 µg/mL. The pyridinyl-substituted adduct **6.23** gave an ID₅₀ value of 2.23 µg/mL, a little more activity than the corresponding oxygen-bridged adducts, but still more active than its aromatised derivative **5.29** which gave a value of >6.25 µg/mL.

The chloroacetyl substituted Diels-Alder adduct **6.25**, and its aromatised derivative **5.11** gave the most promising results. The adduct **6.25** displayed low

anti-tumour activity, with an ID₅₀ value of 38.34 µg/mL. This contrasted well with the two oxygen-bridged analogues, **5.9** and **5.10**, previously tested, which both displayed relatively high activity. The aromatised derivative **5.11**, on the other hand, had an ID₅₀ value of 3.03 µg/mL. This result indicates that **6.25**, with a 12-fold lower activity than its derivative **5.11**, is a promising lead as a prodrug.

These studies have shown that in some cases the extra stability of a sulfur-bridge may offer benefits over an oxygen-bridge, by decreasing the anti-tumour activity of the bridged adduct relative to its aromatised derivative.

6.5 Conclusion

The enhanced stability of isobenzothiophenes relative to isobenzofurans was shown to benefit Diels-Alder chemistry. It was demonstrated that 1-methylsulfanyl-isobenzothiophene, unsubstituted at the 3-position, could react with *N*-aryl maleimides to give Diels-Alder adducts, whereas no such adducts had been isolated in the corresponding isobenzofuran case. However, like the corresponding isobenzofurans, Diels-Alder chemistry was observed to occur more efficiently with alkyl or aryl substitution at the 3-position of the isobenzothiophene. The Diels-Alder chemistry of 1-methylsulfanyl-3-phenyl-isobenzothiophene in particular was investigated. It was demonstrated that a range of hydrogen-bonding functional groups could be introduced into sulfur-bridged adducts via *N*-aryl maleimide based dienophiles. The introduction of an alkylating group into sulfur-bridged adducts via an *N*-aryl maleimide based dienophile was also demonstrated. The aromatised derivative of this adduct displayed promising biological activity, providing an interesting lead for the development of anti-cancer prodrugs.

*DIELS-ALDER APPROACHES
TO ANTI-CANCER PRODRUGS*

Part B:
**Towards Biologically
Active Prodrugs**

Conclusion

It was demonstrated that biological functionality could be introduced into Diels-Alder adducts via the dienophile, and that the bridge cleavage of such adducts could lead to planar aromatic products. The generation of both oxygen-bridged and sulfur-bridged adducts was demonstrated; the resulting increase in stability of the former proving advantageous in many instances. A variety of functionalised adducts were prepared. The introduction of an alkylating functionality into a sulfur-bridged Diels-Alder adduct offered a promising lead into the synthesis of prodrugs for anti-cancer chemotherapy. Such prodrug-based strategies may lead to treatments which offer advantages over existing forms of cancer chemotherapy.

Chapter 7

EXPERIMENTAL

7.1 General

Radial silica chromatography was performed on 1,2, or 4 mm silica plates. The silica was gradient eluted from 0% to 100% of the most polar solvent, and the plates washed and reactivated with methanol.

Melting points (m.p.) were determined on a Reichert Hotstage microscope and are uncorrected.

Elemental microanalyses of crystalline samples were performed at the Chemistry Department of the University of Otago, New Zealand.

Proton nuclear magnetic resonance (^1H NMR) experiments were performed on a Varian Unity 300 MHz Fourier transform spectrometer. Peaks are quoted in parts per million (ppm, δ) relative to tetramethylsilane (TMS). Multiplicities are quoted as singlet (s), broad singlet (br.s.), doublet (d), triplet (t), quartet (q), or multiplet (m). Coupling constants (J) are quoted in Hertz (Hz).

Carbon-13 nuclear magnetic resonance (^{13}C NMR) experiments were performed at 75 MHz on a Varian Unity 300 MHz or Varian XL 300 MHz Fourier transform spectrometer. Peaks are quoted in ppm (δ) relative to TMS.

^1H NMR spectra were referenced to TMS (0.00ppm), deuterated chloroform (CDCl_3 , 7.24ppm), deuterated methanol (CD_3OD , 3.30ppm), d_6 -acetone (2.17ppm), deuterium oxide (D_2O , 4.70ppm), or d_6 -dimethylsulfoxide (2.60ppm).

^{13}C NMR were referenced to CDCl_3 (77.00ppm).

Electron impact (EI) and fast atom bombardment (FAB) mass spectra (m/z) experiments were performed on a Kratos MS80RFA mass spectrometer. High resolution mass results have been compared with expected calculated values.

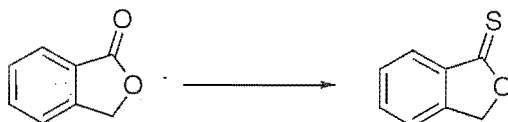
Infra-red (IR) experiments were performed on a Shimadzu FTIR-8201PC or a Perkin Elmer 1600-FTIR spectrophotometer. The sample was presented as either a CDCl_3 solution in a 0.1 or 1.0mm NaCl solution cell, as an oil, or as a Nujol® mull on NaCl plates. Absorption maxima (ν_{max}) are recorded in wavenumbers (cm^{-1}).

Solvents and reagents used were dried and purified in accordance with the recommendations collected in Perrin and Armarego, or as obtained from commercial sources.⁸¹

Part A Experimental

7.2 Chapter 2 Experimental

Preparation of 1(3H)-isobenzofuranthione 2.5²⁰



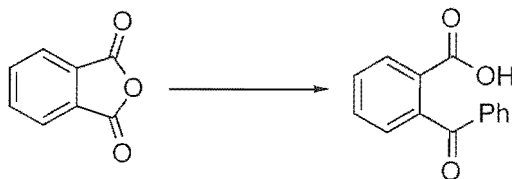
1(3H)-Isobenzofuranone (1.164g, 8.68mmol.) and Lawesson's reagent (1.756g, 4.34mmol.) were refluxed in toluene (25mL) for 17 hours during which time the solution turned from yellow to brown. The toluene was removed under reduced pressure, and the crude solid taken up in ether and filtered to remove ether insoluble components. From this solution a yellow solid precipitated which was recrystallised from ether to give 1(3H)-isobenzofuranthione as yellow needles (590mg, 45%).

m.p. 106-109°C (lit. 106-109°C).²⁰

¹H NMR (CDCl₃) δ 5.59 (s, 2H, OCH₂), 7.50-7.52 (m, 1H, aryl CH).

m/z (EI) 150 (M⁺, 100%), 121 (M-CHO⁺), 89 (M-CHOS⁺).

IR ν_{max} (KBr) 2958, 1315, 1275, 1165, 769 cm⁻¹.

Preparation of 2-benzoylbenzoic acid 2.7²⁵

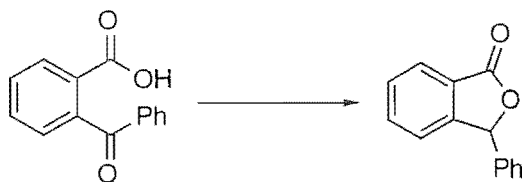
Crushed anhydrous aluminium chloride (32.0g, 240mmol.) was added slowly to a solution of phthalic anhydride (16.6g, 109mmol.) in benzene (66mL), causing HCl gas to be evolved. The reaction was refluxed until completion. Ice was added to the hot brown solution until it turned white, when it was acidified with concentrated HCl (30mL) to give a solid which was filtered and washed with ice water. This solid was neutralised with sodium carbonate solution (10%, 100mL) and impurities removed by boiling with decolourising charcoal (2g). The filtrate was acidified with concentrated HCl (15mL) and the resulting oil crystallised on cooling. The crystals were washed with cold water, dissolved in toluene (110mL) and dried (MgSO₄). The filtrate was concentrated under reduced pressure and petroleum ether (20mL) added to induce crystallisation. 2-Benzoylbenzoic acid was obtained as glassy white needles (17.7g, 33%).

m.p. 126-127°C (lit. 128°C).²⁵

¹H NMR (CDCl₃) δ 7.33-7.40 (m, 1H, aryl CH), 7.43 (m, 1H, aryl CH), 7.45 (m, 1H, aryl CH), 7.56-7.79 (m, 2H, aryl CH), 7.66-7.69 (m, 1H, aryl CH), 7.71-7.74 (m, 2H, aryl CH), 8.10 (d, 1H, J=7.5Hz, aryl CH).

m/z (EI) 226 (M⁺), 182, 149 (C₈H₆O₃⁺), 105 (C₇H₆O⁺).

IR ν_{max} (KBr) 3024, 1675, 1304, 1256 cm⁻¹.

Preparation of 3-phenyl-1(3H)-isobenzofuranone 2.8²⁶

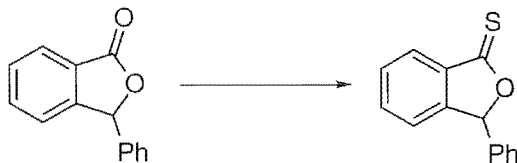
Anhydrous 2-benzoylbenzoic acid (11.46g, 51mmol.), glacial acetic acid (89mL, 1.554mol.), water (23mL), and zinc dust (22.8g, 349mmol.) were refluxed together for 2 hours. The hot supernatant was decanted and left overnight during which time crystallisation of the crude product occurred. The crystals were collected in a Buchner funnel and the remainder of the product precipitated out of the filtrate on addition of water. The crude product was then neutralised with a solution of saturated sodium bicarbonate and collected in a Buchner funnel. This was recrystallised from methanol to give 3-phenyl-1(3H)-isobenzofuranone as large rectangular white crystals (6.2g, 59%).

m.p. 114-116°C (lit. 114-116°C).²⁶

¹H NMR (CDCl₃) δ 6.57 (s, 1H, CH), 7.28 (m, 2H, aryl CH), 7.37-7.41 (m, 4H, aryl CH), 7.61 (m, 1H, aryl CH), 7.73 (m, 1H, aryl CH), 7.93 (d, 1H, J=7.3Hz, aryl CH).

m/z (EI) 210 (M⁺), 165, 105 (C₇H₆O⁺).

IR ν_{max} (KBr) 3032, 1746, 1068, 741 cm⁻¹.

Preparation of 3-phenyl-1(3H)-isobenzofuranthione 2.9²⁰

3-Phenyl-1(3H)-isobenzofuranone (2.519g, 12.0mmol.) and Lawesson's reagent (3g, 7.42mmol.) were refluxed in toluene (30mL) for 17 hours during

which time the solution turned from yellow to red/brown. The toluene was removed under reduced pressure, and the crude solid taken up in ether and filtered to remove the ether-insoluble components. This was recrystallised from ether to give 3-phenyl-1(3H)-isobenzofuranthione as yellow plate crystals (1.357g, 50%).

m.p. 98-100°C (lit. 97-99°C).²⁰

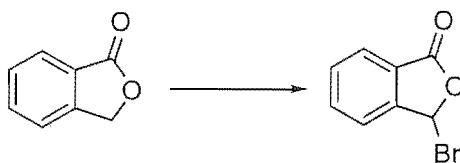
¹H NMR (CDCl₃) δ 6.65 (s, 1H, CH), 7.27-7.40 (m, 5H, aryl CH), 7.52-7.69 (m, 3H, aryl CH), 8.10 (d, 1H, aryl CH).

m/z (EI) 226 (M⁺), 197, 193.

High Res. C₁₄H₁₀SO calc. 226.04523; found 226.0445.

IR ν_{max} (KBr) 3030, 1316, 1264, 1164, 1099 cm⁻¹.

Preparation of 3-bromo-1(3H)-Isobenzofuranone 2.10²⁷



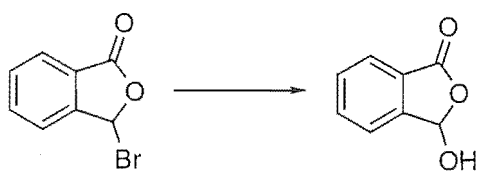
1(3H)-Isobenzofuranone (48.8g, 364mmol.) was heated on an oil bath to 140°C and dry bromine (19.5mL, 381mmol.) was bubbled in on a stream of dry carbon dioxide at such a rate that no excess bromine vapour was observed. The oil bath was maintained at 140-155°C and the carbon dioxide flow was continued for 30 minutes after all the bromine had been added, which took 10 hours. Excess hydrogen bromide was removed by heating at 120°C under reduced pressure. Distillation under reduced pressure gave a fore-run of 1(3H)-isobenzofuranone, followed by 3-bromo-1(3H)-isobenzofuranone, as a clear liquid which solidified to an off-white solid. This was used in the subsequent hydrolysis without further purification (20.5g, 26%).

m.p. 74-75°C (lit. 75°C).²⁷

^1H NMR (CDCl_3) δ 5.34 (s, 1H, CBrH), 7.53 (t, 1H, $J=7.8\text{Hz}$, aryl CH), 7.66 (t, 1H, $J=7.8\text{Hz}$, aryl CH), 7.79 (d, 1H, $J=7.4\text{Hz}$, aryl CH), 7.93 (d, 1H, $J=7.4\text{Hz}$, aryl CH).

m/z (EI) 214 (M^+), 133 ($\text{M}-\text{Br}^+$), 105, 77, 50.

Preparation of 3-hydroxy-1(3H)-isobenzofuranone 2.11²⁸



3-Bromo-1(3H)-isobenzofuranone (20.5g, 96mmol.) was covered with water (80mL) and stirred and heated on a steam bath until the layer of 3-bromo-1(3H)-isobenzofuranone had disappeared. 3-Hydroxy-1(3H)-isobenzofuranone solidified on cooling (0°C) and was recrystallised from hot water as white crystals (14.3g, 99%).

m.p. $93-94^\circ\text{C}$ (lit. 96°C).²⁸

^1H NMR (CDCl_3) δ 6.65 (s, 1H, C(OH)H), 7.64 (m, 2H, aryl CH), 7.72 (d, 1H, $J=7.3\text{Hz}$, aryl CH), 7.89 (d, 1H, $J=7.3\text{Hz}$, aryl CH).

m/z (EI) 150 (M^+), 122 ($\text{M}-\text{CO}^+$), 105, 77, 50.

Preparation of 3-methyl-1(3H)-isobenzofuranone 2.14a²⁹



Magnesium turnings (2.14g, 88mmol.) and methyl iodide (11.2g, 4.9mL, 77mmol.) dissolved in dry ether (50mL) were mixed together under nitrogen. 3-Hydroxy-1(3H)-isobenzofuranone (5g, 33mmol.) dissolved in dry THF (50mL)

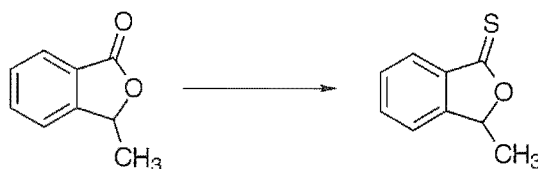
was added dropwise. After 2 hours the reaction was cooled (0°C) and 10% HCl solution (50mL) was added. Stirring at 40°C was continued for 1 hour. The solution was extracted with ether and the organic extracts combined and dried (MgSO₄). The solvent was removed under reduced pressure to give 3-methyl-1(3H)-isobenzofuranone as a yellow oil which was purified by radial silica chromatography (3.3g, 67%).

¹H NMR (CDCl₃) δ 1.64 (d, 3H, J=6.9Hz, CH₃), 5.59 (q, 1H, J=6.4Hz, CH), 7.47-7.56 (m, 2H, aryl CH), 7.7 (t, 1H, J=7.8Hz, aryl CH), 7.88 (d, 1H, J=7.8Hz, aryl CH).

m/z (EI) 148.1 (M⁺), 133.0 (M-CH₃⁺), 105.0 (M-C₂H₄O⁺), 77.0 (M-C₃H₄O₂⁺).

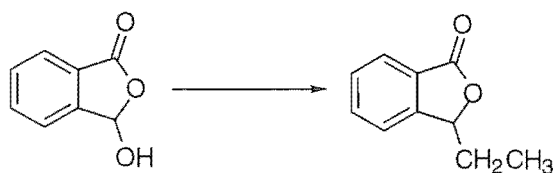
High Res. C₁₂H₈O₂ calc. 148.05243; found 148.05231.

Preparation of 3-methyl-1(3H)-isobenzofuranthione 2.15a



3-Methyl-1(3H)-isobenzofuranone (485mg, 3.3mmol.) was dissolved in toluene (10mL). Lawesson's reagent (662mg, 1.7mmol.) was added and the mixture refluxed overnight. The toluene was removed under reduced pressure and the resultant oil was dissolved in dry ether and filtered through cotton wool 3 times. The ether was removed under reduced pressure to give a red oil which was purified by radial silica chromatography to give 3-methyl-1(3H)-isobenzofuranthione as a yellow oil (309mg, 57%).

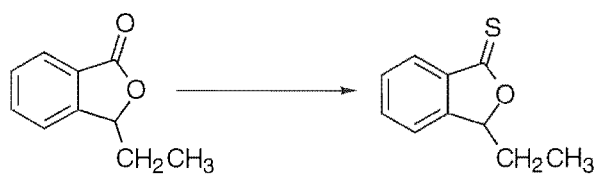
¹H NMR (CDCl₃) δ 1.72 (d, 3H, J=6.8Hz, CH₃), 5.83 (q, 1H, J=6.8Hz, CH), 7.43 (d, 1H, J=6.5Hz, aryl CH), 7.52 (t, 1H, J=7.8Hz, aryl CH), 7.68 (t, 1H, J=6.5Hz, aryl CH), 8.04 (d, 1H, J=7.8Hz, aryl CH).

Preparation of 3-ethyl-1(3H)-isobenzofuranone 2.14b²⁹

Magnesium turnings (854mg, 29.3mmol.) and ethyl bromide (1.96mL, 26.3mmol.) dissolved in dry ether (25mL) were mixed together under nitrogen. 3-Hydroxy-1(3H)-isobenzofuranone (2g, 11.2mmol.) dissolved in dry THF (25mL) was added dropwise. After 2 hours it was cooled to 0°C and hydrolysed with 10% HCl solution. This reaction mixture was stirred at 40°C for another hour. The solution was extracted with ether and the organic extracts combined and dried (MgSO₄). The solvent was removed under reduced pressure to give 3-ethyl-1(3H)-isobenzofuranone as a yellow oil which was purified by radial silica chromatography (408mg, 22%).

¹H NMR (CDCl₃) δ 1.00 (t, 3H, J=7.4Hz, CH₃), 1.84 (m, 1H, CH₂), 2.13 (m, 1H, CH₂), 5.47 (m, 1H, CH), 7.47 (d, 1H, J=8.8Hz, aryl CH), 7.53 (t, 1H, J=7.3Hz, aryl CH), 7.69 (t, 1H, J=7.3Hz, aryl CH), 7.69 (t, 1H, J=7.3Hz, aryl CH), 7.89 (d, 1H, J=7.3Hz, aryl CH).

m/z (EI) 162.1 (M⁺), 133.0 (M-C₂H₅⁺).

Preparation of 3-ethyl-1(3H)-isobenzofuranthione 2.15b

3-Ethyl-1(3H)-isobenzofuranone (328mg, 2.0mmol.) was dissolved in toluene (10mL) and Lawesson's reagent (409mg, 1.0mmol.) added. This mixture was refluxed overnight in an oil bath. The toluene was removed under reduced pressure

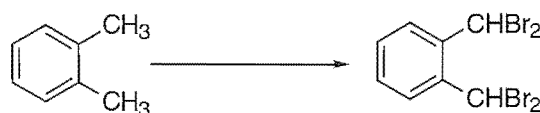
and the resultant oil was dissolved in dry ether and filtered through cotton wool. This was repeated 3 times. The ether was removed under reduced pressure to give a red/brown oil. This was purified by radial silica chromatography to give 3-ethyl-1(3H)-isobenzofuranthione as a yellow oil (340mg, 94%).

^1H NMR (CDCl_3) δ 1.01 (t, 3H, $J=7.3\text{Hz}$, CH_3), 1.91 (m, 1H, CH_2), 2.19 (m, 1H, CH_2), 5.72 (m, 1H, CH), 6.99-7.07 (m, 2H, aryl CH), 7.91-8.13 (m, 2H, aryl CH).

m/z (EI) 178.1 (M^+), 149 ($\text{M}-\text{C}_2\text{H}_5^+$).

High Res. $\text{C}_{10}\text{H}_{10}\text{SO}$ m/z calc. 178.04528; found 178.04499.

Preparation of 1,2-bis-(dibromomethyl)-benzene 2.17³¹



Dry 2-xylene (29.25g, 19.48mL, 275mmol.) was stirred and heated to 120°C. A lamp was placed close to the flask so as to admit the maximum amount of light. Bromine (175g, 1.1mol.) was added in portions from a dropping funnel to the reaction flask at such a rate that the bromine colour was removed as fast as it was added. After approximately one-half of the bromine was added the temperature was slowly increased to 175°C for the remainder of the addition. After 12 hours all the bromine had been added and the mixture was illuminated and stirred at 170°C for a further hour. The mixture was cooled to room temperature and crystallised out overnight as a dark solid. This was dissolved in hot, dry chloroform (500mL) and treated with Norit (25g). The mixture was filtered, the Norit washed with hot chloroform, and the Norit treatment repeated with another portion (25g). The filtrate was concentrated under reduced pressure and chilled (0°C), to give a pale brown solid which was collected and washed with cold chloroform (5mL). The filtrate was concentrated further to obtain further product. Recrystallisation from

chloroform gave 1,2-bis(dibromomethyl) benzene as off-white crystals (52.85g, 46%).

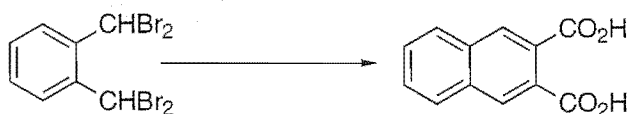
m.p. 112-116°C (lit. 115-116°C).

^1H NMR (CDCl_3) δ 7.14 (s, 2H, CHBr_2), 7.38 (m, 2H, aryl CH), 7.67 (m, 2H, aryl CH).

m/z (EI) 422 (M^+), 341 ($\text{M}-\text{Br}^+$), 262 ($\text{M}-\text{Br}_2^+$), 181 ($\text{M}-\text{Br}_3^+$), 102 ($\text{M}-\text{Br}_4^+$).

IR ν_{max} 1315.4, 1236.3, 1170.7, 1137.9 cm^{-1} .

Preparation of naphthalene-2,3-dicarboxylic acid 2.18³³



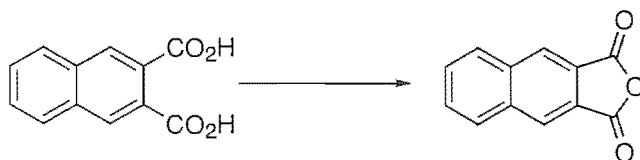
A mixture of 1,2-bis(dibromomethyl)-benzene (20.0g, 47.4mmol.), sodium iodide (47.4g, 316mmol.), maleic anhydride (14.2g, 145mmol.), and dry DMF (150mL) was stirred at 60-70°C for 5 hours. The reaction mixture was poured into water (1.5L) containing sodium bisulfite (20.0g, 192mmol.) and the resulting precipitate collected on a Buchner funnel to give naphthalene-2,3-dicarboxylic acid as prisms (7.26g, 71%).

m.p. 234-235°C (lit. 238-239°C).

^1H NMR (D_2O) δ 7.57 (m, 2H, aryl CH), 7.91 (m, 2H, aryl CH), 8.15 (s, 2H, CHCCO_2).

m/z (EI) 198 ($\text{M}-\text{H}_2\text{O}^+$), 154, 126.

IR ν_{max} (Nujol) 1698, 1460, 1377, 1223, 1140 cm^{-1} .

Preparation of naphthalene-2,3-dicarboxylic anhydride 2.19

Naphthalene-2,3-dicarboxylic acid (7.82g, 36.2mmol.) was refluxed in freshly distilled acetic anhydride (50mL, 530mmol.) for 3 hours. The remaining acetic anhydride was then removed under reduced pressure to give naphthalene-2,3-dicarboxylic anhydride as a white crystalline solid (7.50g, 100%)

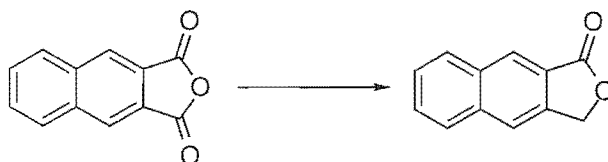
m.p. 241-244°C.

^1H NMR (CDCl_3) δ 7.83 (m, 2H, aryl CH), 8.15 (m, 2H, aryl CH), 8.56 (s, 2H, aryl CH).

m/z (EI) 198 (M^+), 154 ($\text{M}-\text{CO}_2^+$), 126, 63.

High Res. $\text{C}_8\text{H}_6\text{O}_3$ m/z calc. 198.03170; found 198.03170.

IR ν_{max} (Nujol) 2360, 1836, 1763, 1460, 1377, 1247, 1198 cm^{-1} .

Preparation of 1,3-dihydronaphtho[2,3-*c*]furan-1-one 2.21

Sodium borohydride (20mg, 0.529mmol.) in dry DMF (1mL) was stirred at 0°C while naphthalene-2,3-dicarboxylic anhydride (100mg, 0.505mmol.) in DMF (4mL) was added over 5 minutes. Stirring was continued for one hour at room temperature. Hydrochloric acid (6M, 1mL) was added cautiously, followed by water (10mL) and the mixture extracted with ether. The ether fractions were combined, dried (MgSO_4), and the ether removed under reduced pressure to give naphthalide as small white needles (60.8mg, 65%).

m.p. 205-206°C.

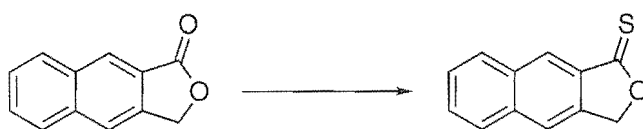
^1H NMR (CDCl_3) δ 5.50 (s, 2H, CH_2), 7.65 (m, 2H, aryl CH), 7.95 (m, 2H, aryl CH), 8.06 (d, 1H, $J=7.4\text{Hz}$, aryl CH), 8.52 (s, 1H, COCCH).

m/z (EI) 184 (M^+), 140 ($\text{M}-\text{CO}_2^+$).

High Res. $\text{C}_{12}\text{H}_8\text{O}_2$ m/z calc. 184.05246; found 184.05253.

IR ν_{max} (Nujol) 1757, 1690, 1462, 1377, 1300, 1102, 1031, 783, 746 cm^{-1} .

Preparation of 1,3-dihydronaphtho[2,3-*c*]furan-1-thione 2.22



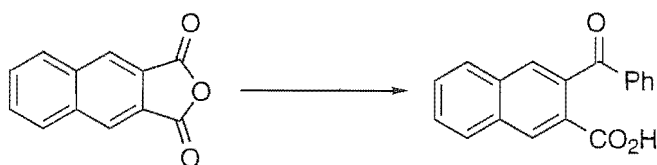
Naphthalene-2,3-dicarboxylic anhydride (60mg, 0.326mmol.) and Lawesson's reagent (66mg, 0.163mmol.) were refluxed in toluene (5mL) for 17 hours. The toluene was removed under reduced pressure to give an oil. This was purified by radial silica chromatography to give 1,3-dihydronaphtho[2,3-*c*]furan-1-thione as a yellow solid (9.3mg, 14%)

m.p. 193-194°C.

^1H NMR (CDCl_3) δ 5.75 (s, 2H, CH_2), 7.59-7.68 (m, 2H, aryl CH), 7.93 (m, 2H, aryl CH), 8.09 (d, 1H, $J=8.3\text{Hz}$, aryl CH), 8.66 (s, 1H, CSCCH).

m/z (EI) 200 (M^+), 140 ($\text{M}-\text{CSO}^+$).

High Res. $\text{C}_{12}\text{H}_8\text{SO}$ m/z calc. 200.02962; found 200.02936.

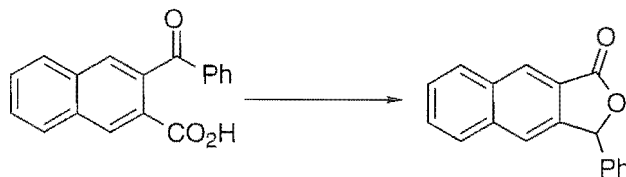
Preparation of 3-benzoylnaphthoic acid 2.23

Crushed anhydrous aluminium chloride (19.28g, 145mmol.) was added slowly to a solution of 2,3-naphthalic anhydride (10g, 50mmol.) in benzene (40mL) causing hydrogen chloride gas to be evolved. The reaction was refluxed until no more gas was evolved. Ice was then added until the solution turned from brown to white, whereupon it was acidified with concentrated hydrochloric acid. The resulting solid was filtered and washed with cold water. This solid was neutralised with sodium carbonate solution (10%, 31.3mL, 0.030mmol.) and the impurities were removed by boiling with decolourising charcoal (625mg). The mixture was filtered and the filtrate acidified with concentrated hydrochloric acid (5mL) to give an oil which crystallised on cooling. The crystals were washed with cold water and dried under reduced pressure to give 3-benzoylnaphthoic acid as white crystals (9.04g, 65%).

m.p. 202-204°C.

m/z (EI) 276.1 (M^+), 232.1 ($M-CO_2^+$), 155.0, 105.0.

High Res. $C_{18}H_{12}O_3$ m/z calc. 276.07864; found 276.07917.

Preparation of 3-phenyl-1(3H)-isonaphtho[2,3-c]furan-1-one 2.24

3-Benzoyl phthalic acid (9.04g, 33mmol.), zinc dust (18.1g, 277mmol.), glacial acetic acid (18.1g, 301mmol.), and water (20mL) were refluxed together for

3 hours. The hot supernatant was decanted and left to crystallise overnight to give the crude lactone. The crystals were collected on a Buchner funnel and the remainder of the lactone precipitated out of the filtrate on addition of water. The crude lactone was then neutralised with a saturated solution of sodium bicarbonate and collected on a Buchner funnel. Recrystallisation from ethanol gave 3-phenyl-1(3H)-isonaphtho[2,3-c]furan-1-one as white crystals (1.6g, 19%).

m.p. 151-152°C.

Analysis $C_{18}H_{12}O_2$ calc. C, 83.06; H, 4.65; found: C, 82.72; H, 4.50.

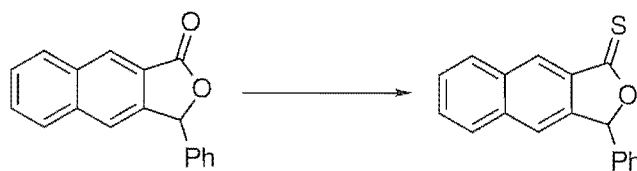
1H NMR ($CDCl_3$) δ 6.58 (s, 1H, CH), 7.33-7.41 (m, 5H, aryl CH), 7.59-7.66 (m, 2H, aryl CH), 7.75 (s, 1H, aryl CH), 7.87 (d, 1H, $J=7.3$ Hz, aryl CH), 8.07 (d, 1H, $J=7.3$ Hz, aryl CH), 8.55 (s, 1H, aryl CH).

m/z (EI) 260 (M^+), 216 ($M-CO_2^+$).

High Res. $C_{18}H_{12}O_2$ m/z calc. 260.08378; found 260.08378.

Preparation of 3-phenyl-1(3H)-isonaphtho[2,3-c]furan-1-thione

2.25



3-Phenyl-1(3H)-isonaphtho[2,3-c]furan-1-one (300mg, 1.15mmol.) and Lawesson's reagent (300mg, 0.742mmol.) were refluxed in toluene (15mL) for 17 hours during which time the solution turned from yellow to brown. The toluene was removed under reduced pressure, and the crude solid taken up in ether and filtered to remove the ether-insoluble components. This was recrystallised from ether to give 3-phenyl-1(3H)-isonaphtho[2,3-c]furan-1-thione as pale yellow crystals (144mg, 46%).

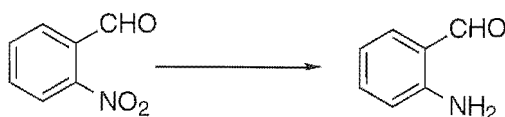
m.p. 142-144°C.

^1H NMR (CDCl_3) δ 6.81 (s, 1H, CH), 7.31-7.43 (m, 5H, aryl CH), 7.56-7.647 (m, 2H, aryl CH), 7.74 (s, 1H, aryl CH), 7.86 (d, 1H, $J=7.8\text{Hz}$, aryl CH), 8.09 (d, 1H, $J=7.8\text{Hz}$, aryl CH), 8.68 (s, 1H, aryl CH).

m/z (EI) 276 (M^+), 216.

High Res. $\text{C}_{18}\text{H}_{12}\text{SO}$ m/z calc. 276.06094; found 276.06108.

Preparation of 2-aminobenzaldehyde 2.29³⁵



Water (175mL), ferrous chloride heptahydrate (105g, 380mmol.), concentrated hydrochloric acid (0.5mL), and 2-nitrobenzaldehyde (6g, 40mmol.) were stirred and heated together to 90°C , then ammonium hydroxide (25mL, 642mmol.) was added. Three further portions of ammonium hydroxide (10mL, 257mmol.) were added at 2 minute intervals. Immediately after the addition of the last portion of ammonium hydroxide the reflux condenser was removed and the reaction vessel connected to a steam distillation assembly. The reaction mixture was steam-distilled as rapidly as possible, collecting two 250mL fractions over a period of 15 minutes. The distillate was saturated with sodium chloride and cooled to 0°C resulting in the formation of a precipitate. This was collected in a Buchner funnel to give 2-aminobenzaldehyde as a bright yellow solid (2.82g, 58%).

m.p. $39-40^\circ\text{C}$ (lit. $38-39^\circ\text{C}$).³⁵

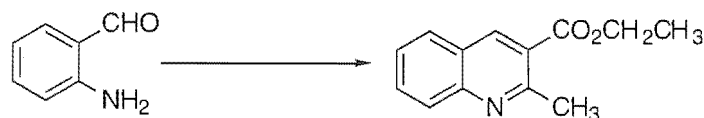
^1H NMR (CDCl_3) δ 6.66 (d, 1H, $J=8.3\text{Hz}$, aryl CH), 6.76 (t, 1H, $J=7.8\text{Hz}$, aryl CH), 7.32 (t, 1H, $J=7.4\text{Hz}$, aryl CH), 7.49 (d, 1H, $J=7.8\text{Hz}$, aryl CH), 9.88 (s, 1H, CHO).

m/z (EI) 121.1 (M^+), 93.1 ($\text{M}-\text{CO}^+$).

High Res. $\text{C}_7\text{H}_7\text{NO}$ m/z calc. 121.05276; found 121.05260.

IR ν_{\max} (Nujol) 2360, 1651, 15778, 1529, 1458, 1377, 1346 cm^{-1} .

Preparation of 3-ethoxycarbonyl-2-methyl-quinoline 2.30³⁶

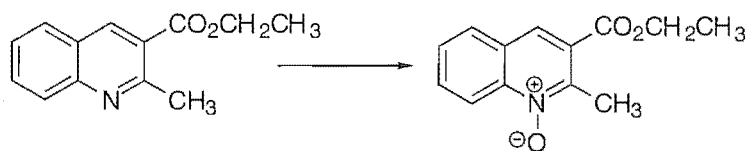


2-Aminobenzaldehyde (2.82g, 23mmol.) was dissolved in the minimum amount of ethanol and ethyl acetoacetate (2.96mL, 23mmol.) added dropwise. A sodium hydroxide pellet was added and the solution stirred overnight. The ethanol was removed under reduced pressure to give an oil which was partitioned between ethyl acetate and water. The organic fractions were combined, dried (MgSO_4) and the ethyl acetate removed under reduced pressure to give a solid which was recrystallised from ethanol to give 3-ethoxycarbonyl-2-methyl-quinoline as pale orange needles (1.82g, 38%).

m.p. 69-70°C (lit. 71-72°C).

^1H NMR (CDCl_3) δ 1.46 (t, 3H, $J=7.3\text{Hz}$, CH_3), 3.00 (s, 3H, aryl CH_3), 4.45 (q, 2H, $J=7.4\text{Hz}$, OCH_2), 7.55 (t, 1H, $J=7.8\text{Hz}$, aryl CH), 7.79 (t, 1H, $J=7.3\text{Hz}$, aryl CH), 7.88 (d, 1H, $J=7.8\text{Hz}$, aryl CH), 8.04 (d, 1H, $J=7.8\text{Hz}$, aryl CH), 8.75 (s, 1H, COCCH).

IR ν_{\max} 3018.4, 1720.4, 1287.7, 1236.3, 1207.4, 1197.7, 1064.6, 908.4 cm^{-1} .

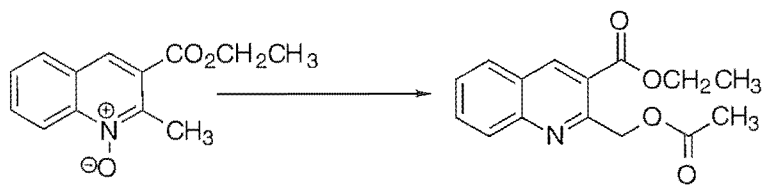
Preparation of 3-ethoxycarbonyl-2-methyl-quinoline *N*-oxide 2.31

3-Ethoxycarbonyl-2-methyl-quinoline (150mg, 0.697mmol.) was dissolved in dichloromethane (5mL) and 3-chloroperbenzoic acid (360mg, 2.09mmol.) was added. This was stirred at room temperature and monitored by TLC. After one hour the starting material was no longer visible by TLC. The mixture was partitioned between dichloromethane and saturated aqueous sodium bicarbonate solution. The dichloromethane fractions were combined, dried (MgSO₄), and the dichloromethane removed under reduced pressure to give a solid. This was purified by radial silica chromatography to give 3-ethoxycarbonyl-2-methyl-quinoline *N*-oxide as a brown solid (146mg, 91%).

m.p. 45-47°C.

¹H NMR (CDCl₃) δ 1.47 (t, 3H, J=6.8Hz, CH₃), 2.99 (s, 3H, aryl CH₃), 4.47 (q, 2H, J=7.3Hz, OCH₂), 7.67 (t, 1H, J=6.8Hz, aryl CH), 7.91 (m, 2H, aryl CH), 8.29 (s, 1H, COCCH), 8.80 (d, 1H, J=8.8Hz, aryl CH).

IR ν_{max} 1724.2, 1321.1, 1288.4, 1240.1, 1105.1, 1051.1, 908.4 cm⁻¹.

Preparation of 2-acetoxymethyl-3-ethoxycarbonyl-quinoline 2.32

Acetic anhydride (1.09mL, 11.6mmol.) was added to 3-ethoxycarbonyl-2-methyl-quinoline *N*-oxide (1.33g, 5.75mmol.) in benzene (15mL). This was refluxed for 3 hours. The reaction mixture was then distilled under reduced

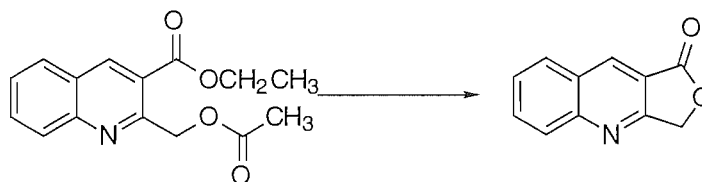
pressure and any remaining solvent removed under reduced pressure. The solid formed was then dissolved in ethyl acetate and partitioned with aqueous sodium bicarbonate solution. The ethyl acetate fractions were combined, dried (MgSO_4), and the ethyl acetate removed under reduced pressure to give 2-acetoxymethyl-3-ethoxycarbonyl-quinoline as a pale yellow solid (1.45g, 93%).

m.p. 68-70°C.

^1H NMR (CDCl_3) δ 1.46 (t, 3H, $J=7.3\text{Hz}$, CH_3), 2.23 (s, 3H, COCH_3), 4.46 (q, 2H, $J=6.9\text{Hz}$, OCH_2CH_3), 5.74 (s, 2H, OCH_2), 7.61 (t, 1H, $J=8.3\text{Hz}$, aryl CH), 7.82 (t, 1H, $J=8.3\text{Hz}$, aryl CH), 7.91 (d, 1H, $J=8.2\text{Hz}$, aryl CH), 8.11 (d, 1H, $J=8.3\text{Hz}$, aryl CH), 8.83 (s, 1H, COCCH).

IR ν_{max} 3030.0, 1720.4, 1622.0, 1369.4, 1276.8, 1253.6, 1226.6, 1207.4, 1068.5 cm^{-1} .

Preparation of 4-aza-3H-isonaphtho[c]furan-1-one 2.33



2-Acetoxymethyl-3-ethoxycarbonyl-quinoline (876mg, 3.21mmol.) was dissolved in dichloromethane (5mL) and concentrated hydrochloric acid (1.2mL, 19.2mmol.) added. The solution was then stirred for 3 days to give a brown oily solution which was neutralised cautiously with saturated sodium bicarbonate solution and partitioned with ethyl acetate. The ethyl acetate fractions were combined, dried (MgSO_4), and the ethyl acetate removed under reduced pressure to give an oily solid. This was purified by dissolving the oil in methanol and drawing it off to give a solid which was recrystallised from ethyl acetate and petroleum ether to give 4-aza-3H-isonaphtho[c]furan-1-one as off white crystals (114mg, 17%).

m.p. 186°C (decomp.).

^1H NMR (CDCl_3) δ 5.51 (s, 2H, CH_2), 7.71 (t, 1H, $J=8.3\text{Hz}$, aryl CH), 7.95 (t, 1H, $J=8.3\text{Hz}$, aryl CH), 8.08 (d, 1H, $J=8.3\text{Hz}$, aryl CH), 8.21 (d, 1H, $J=8.3\text{Hz}$, aryl CH), 8.81 (s, 1H, COCCH).

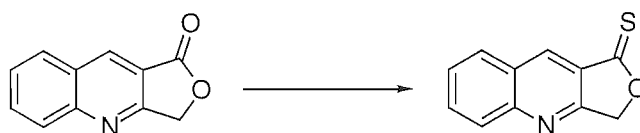
^{13}C NMR (CDCl_3) δ 70.79, 110.67, 117.09, 119.14, 127.41, 127.68, 129.34, 130.02, 133.12, 136.20, 163.49.

m/z (EI) 185.0 (M^+), 156.0, 128.0.

High Res. $\text{C}_{11}\text{H}_7\text{NO}_2$ m/z calc. 185.04768, found 185.04718.

IR ν_{max} 3014.5, 1770.5, 1633.6, 1585.4, 1510.2, 1326.9, 1164.9, 1035.7, 1012.6 cm^{-1} .

Preparation of 4-aza-3H-isonaphtho[c]furan-1-thione 2.34



4-Aza-3H-isonaphtho[c]furan-1-one (98mg, 0.529mmol.) and Lawesson's reagent (107mg, 0.265mmol.) were refluxed in toluene (5mL) for 18 hours. The toluene was removed under reduced pressure to give a brown oil. This was purified by radial silica chromatography to give 4-aza-3H-isonaphtho[c]furan-1-thione as a brown solid (3.4mg, 3%).

m.p. 110-112°C.

^1H NMR (CDCl_3) δ 5.72 (s, 2H, CH_2), 7.70 (t, 1H, $J=8.3\text{Hz}$, aryl CH), 7.94 (t, 1H, $J=7.3\text{Hz}$, aryl CH), 8.08 (d, 1H, $J=8.3\text{Hz}$, aryl CH), 8.19 (d, 1H, $J=7.8\text{Hz}$, aryl CH).

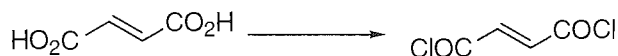
m/z 201 (M^+), 141.

High Res. $\text{C}_{11}\text{H}_7\text{NSO}$ m/z calc. 201.02486; found 201.02501.

IR ν_{max} 1770.5, 1631.7, 1622.0, 1487.0, 1421.4, 1236.3, 1132.1 cm^{-1} .

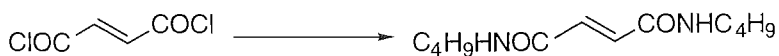
7.3 Chapter 3 Experimental

Preparation of fumaroyl dichloride 3.4⁴⁸



Fumaric acid (10g, 86mmol.) and phosphorus pentachloride (17.94g, 86mmol.) were dissolved in dichloromethane (80mL) and stirred together. The reaction flask was attached to a reflux condenser followed by a calcium chloride drying tube followed by a gas absorption trap. The flask was heated on a water bath with stirring until reaction commenced and then for a further 30 minutes until the evolution of hydrogen chloride gas had ceased. The reaction flask was then attached to distillation apparatus and the water bath exchanged for an oil bath. The oil bath was gradually heated to 200°C to remove phosphorus oxychloride. Then the flask was allowed to cool, the water condenser was replaced by an air condenser and the residual liquid was distilled under reduced pressure (water pump) to give fumaroyl dichloride as a clear oil (12.5g, 95%). This was used immediately in the subsequent reactions.

Preparation of *N,N'*-dibutylfumaramide 3.5



Fumaroyl chloride (12.2g, 80mmol.) was added slowly to a stirred solution of *n*-butylamine (11.7g, 160mmol.) in petroleum ether (90mL) which was cooled in an ice bath. A white mist was produced in addition to an opaque white solution. After the addition was complete, hydrochloric acid (1M, 60mL) was added and the mixture shaken in a separating funnel. The mixture was allowed to separate and

then the organic layer containing the amide was washed with water until acid free. The petroleum ether was removed under reduced pressure and the resulting solid recrystallised from ethanol to give *N,N'*-dibutylfumaramide as fine white crystals (14.5g, 80%).

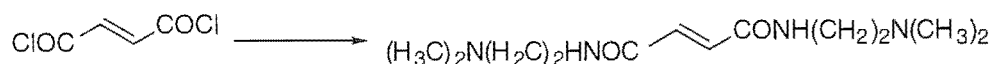
m.p. 270-271°C.

^1H NMR (DMSO) δ 0.97 (t, 6H, $J=7.4\text{Hz}$, CH_3), 1.39 (m, 4H, CH_2CH_3), 1.51 (m, 4H, NHCH_2CH_2), 3.24 (m, 4H, NHCH_2), 6.89 (s, 2H, CH), 8.44 (br.s, 2H, NH).

m/z (EI) 226.2 (M^+), 154.1.

High Res. $\text{C}_{12}\text{H}_{22}\text{N}_2\text{O}_2$ m/z calc. 226.16813; found 226.16787.

Preparation of *N,N'*-di(*N*-dimethylaminoethyl)fumaramide 3.6



Fumaroyl chloride (12.2g, 80mmol.) was added slowly to a stirred solution of *N,N*-dimethylethylenediamine (14.1g, 17.5mL, 160mmol.) in dichloromethane (50mL) which was cooled in an ice bath. A white mist was produced and the solution turned brown. The dichloromethane was removed under reduced pressure to give an oily brown solid. This was recrystallised from ethanol to give a pale brown solid, which was dissolved in distilled water and sodium hydroxide pellets added until pH 14. The mixture was extracted with ethyl acetate, the ethyl acetate fractions were combined, dried (MgSO_4), and the ethyl acetate removed under reduced pressure to give *N,N'*-di(*N*-dimethylaminoethyl)fumaramide as white crystals (2.4g, 12%).

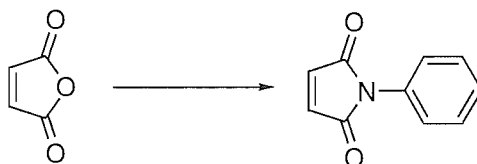
m.p. 178°C (decomp.).

^1H NMR (CDCl_3) δ 2.23 (s, 12H, CH_3), 2.45 (m, 4H, NCH_2), 3.41 (m, 4H, NHCH_2), 6.54 (br.s, 2H, NH), 6.87 (s, 2H, CH).

m/z (EI) 254 (M^+), 71.

High Res. $C_{12}H_{10}N_2O_2$ m/z calc. 254.1743, found 254.1743.

Preparation of *N*-phenyl maleimide⁵⁰



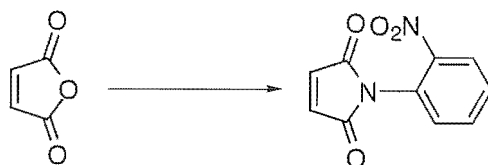
Maleanilic Acid. Maleic Anhydride (20g, 0.2mol.) was stirred in ethyl ether (250mL) until all had dissolved and a solution of aniline (18.6mL, 0.2mol.) in ethyl ether (20mL) was run in through a dropping funnel. The resulting thick suspension was stirred at room temperature for 1 hour and cooled to 15-20°C in an ice bath. The product was obtained by suction filtration to give maleanilic acid as a fine cream coloured powder which was used in the next step without purification.

m.p. 200-202°C (lit. 201-202°C).

N-phenyl maleimide. In a conical flask were placed acetic anhydride (68.4mL, 0.72mol.), anhydrous sodium acetate (6.6g, 0.08mol.), and all the maleanilic acid prepared above. This suspension was dissolved by swirling and heating on a steam bath for 30 minutes. The reaction mixture was then cooled almost to room temperature in a cold water bath and poured into ice water. The precipitated product was removed by suction filtration and washed three times with portions of ice-cold water (10mL) and once with petroleum ether (10mL). Recrystallisation from cyclohexane gave *N*-phenyl maleimide as yellow needles (17.87g, 2 steps 51%).

m.p. 89-90°C (lit. 89-90°C).

1H NMR ($CDCl_3$) δ 6.85 (s, 2H, COCH), 7.33-7.40 (m, 3H, aryl CH), 7.45-7.50 (m, 2H, aryl CH).

Preparation of *N*-(2-nitrophenyl)maleimide 3.7

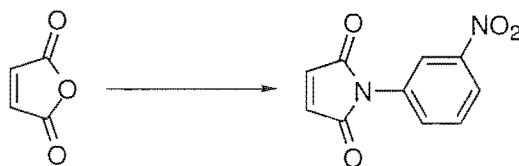
Maleic anhydride (19.6g, 200mmol.) was stirred in ether (250mL) until completely dissolved. 2-Nitroaniline (27.6g, 200mmol.) in ether (250mL) was then added and the resulting solution was stirred for 3 weeks at room temperature during which time precipitation gradually occurred. This solid was refluxed overnight with freshly distilled acetic anhydride (200mL, 2.12mol.). The acetic anhydride was removed under reduced pressure and the resulting solid partitioned between ethyl acetate and water. The ethyl acetate fractions were combined, dried (MgSO_4) and the ethyl acetate removed under reduced pressure to give *N*-(2-nitrophenyl)maleimide as off-white crystals which were recrystallised from ethyl acetate and petroleum ether (26.94g, 2 steps 62%).

m.p. 104-107°C.

^1H NMR (CDCl_3) δ 6.93 (s, 2H, COCH), 7.43 (d, 1H, $J=7.8\text{Hz}$, aryl CH), 7.60 (t, 1H, $J=7.8\text{Hz}$, aryl CH), 7.75 (t, 1H, $J=7.8\text{Hz}$, aryl CH), 8.15 (d, 1H, $J=7.8\text{Hz}$, aryl CH).

m/z (EI) 218 (M^+), 172.0 ($\text{M}-\text{NO}_2^+$).

High Res. $\text{C}_{10}\text{H}_6\text{N}_2\text{O}_4$ m/z calc. 218.03276; found 218.03270.

Preparation of *N*-(3-Nitrophenyl)maleimide 3.8

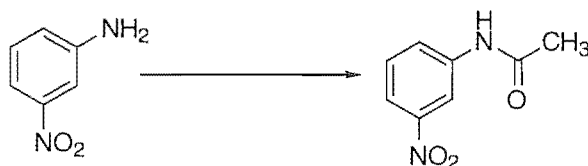
Maleic anhydride (19.6g, 200mmol.) was stirred in ether (250mL) until completely dissolved. 2-Nitroaniline (27.6g, 200mmol.) in ether (250mL) was then added and the resulting solution was stirred overnight at room temperature during which time precipitation occurred. This solid was refluxed overnight with freshly distilled acetic anhydride (200mL, 2.12mol.) during which time the solution turned a dark brown colour. The acetic anhydride was removed under reduced pressure and the resulting solid partitioned between ethyl acetate and water. The ethyl acetate fractions were combined, dried (MgSO₄), and the ethyl acetate removed under reduced pressure to give *N*-(3-nitrophenyl)maleimide as pale brown crystals which were recrystallised from ethyl acetate and petroleum ether (20.28g, 2 steps 47%).

m.p. 106-109°C.

¹H NMR (CDCl₃) δ 6.94 (s, 2H, COCH), 7.66 (t, 1H, J=7.8Hz, aryl CH), 7.79 (d, 1H, J=7.8Hz, aryl CH), 8.24 (d, 1H, J=7.8Hz, aryl CH), 8.34 (s, 1H, aryl CH).

m/z (EI) 218.0 (M⁺), 172.0 (M-NO₂⁺).

High Res. C₁₀H₆N₂O₄ calc. 218.03276; found 218.03291.

Preparation of *N*-acetyl 3-aminonitrobenzene 3.11

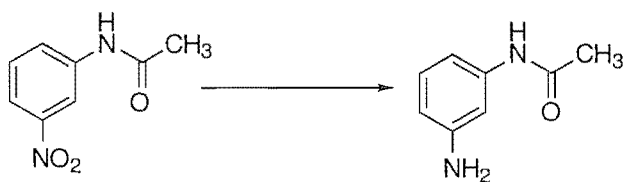
3-Nitroaniline (20g, 145mmol.) was placed in a round-bottomed flask and acetic anhydride was added (50mL, 530mmol.). This was refluxed on an oil bath overnight. The following day the excess acetic anhydride was removed under reduced pressure and the resulting solid was recrystallised from methanol to give *N*-acetyl 3-aminonitrobenzene as pale brown crystals (19g, 73%).

m.p. 149-152°C.

^1H NMR (CDCl_3) δ 2.15 (s, 3H, CH_3), 7.51 (t, 1H, $J=7.8\text{Hz}$, aryl CH), 7.83 (dd, 1H, $J=1.0, 8.3\text{Hz}$, aryl CH), 7.91 (dd, 1H, $J=1.0, 8.3\text{Hz}$, aryl CH), 8.58 (3, 1H, aryl CH).

m/z (EI) 180 (M^+), 138 ($\text{M}-\text{CH}_3\text{CO}^+$), 92.

High Res. $\text{C}_8\text{H}_8\text{NO}_3$ m/z calc. 180.0535; found 180.0533.

Preparation of *N*-acetyl 3-aminoaniline 3.12

N-Acetyl 3-aminonitrobenzene (5g, 27.8mmol.) was placed in a round bottomed flask and dissolved in the minimum amount of methanol. 5% Palladium on carbon (3g) was added and the flask attached via a tap to a hydrogen-filled balloon. With the tap closed the air in the reaction vessel was evacuated under the reduced pressure of a water pump, also connected via a tap. The tap to the water pump was then closed and the tap to the hydrogen balloon opened. The reaction was stirred

and monitored by TLC, with the hydrogen balloon being refilled where necessary. Once the reaction was complete, the reaction mixture was filtered and the solvent removed under reduced pressure to give *N*-acetyl 3-aminoaniline as a white solid (2.5g, 60%)

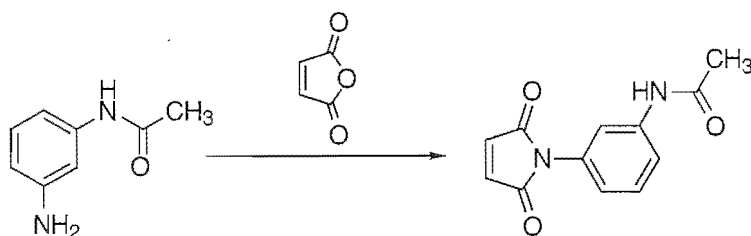
m.p. 61-62°C.

^1H NMR (CDCl_3) δ 2.05 (s, 3H, CH_3), 6.46 (dd, 1H, $J=1.0, 8.7\text{Hz}$, aryl CH), 6.81 (dd, 1H, $J=1.0, 8.8\text{Hz}$, aryl CH), 6.98-7.05 (m, 2H, aryl CH), 9.61 (br.s, 1H, NH).

m/z (EI) 150.1 (M^+), 108.1 ($\text{M}-\text{COCH}_2^+$).

High Res. $\text{C}_8\text{H}_{10}\text{N}_2\text{O}$ m/z calc. 150.07931; found 150.07920.

Preparation of *N*-(3-acetylaminophenyl)maleimide 3.13



N-acetyl 3-aminoaniline (4.8g, 0.032mol.) was dissolved in THF. Maleic anhydride (3.1g, 0.032mol.) in THF was then added to the solution and it was stirred overnight to give a yellow precipitate. To this precipitate, sodium acetate (1.5g, 0.023mol.) and acetic anhydride (10mL, 106mmol.) were added. The flask was heated on a steam bath until completely dissolved and then for a further 30 minutes. The resulting yellow solution was then extracted with dichloromethane. The organic fractions were combined, dried (MgSO_4), and the solvent removed under reduced pressure to give a solid which was recrystallised from ethyl acetate to give *N*-(3-acetylaminophenyl)maleimide as yellow needles (1.36g, 19%).

m.p. 188-190°C.

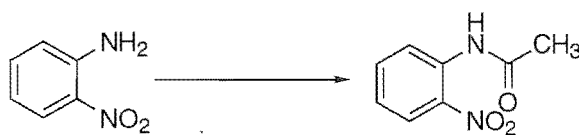
Analysis $C_{12}H_{10}N_2O_3$ calc. C, 62.60; H, 4.38; N, 12.17; found C, 62.57; H, 4.35; N, 12.25.

1H NMR ($CDCl_3$) δ 2.17 (s, 3H, CH_3), 6.86 (s, 2H, COCH), 7.09 (d, 1H, $J=7.8Hz$, aryl CH), 7.35-7.49 (m, 2H, aryl CH), 7.59 (s, 1H, aryl CH).

m/z (EI) 230.1 (M^+), 188.1 ($M-COCH_2^+$).

High Res. $C_{12}H_{10}N_2O_3$ m/z calc. 230.06914; found 230.06925.

Preparation of *N*-acetyl 2-aminonitrobenzene



2-Nitroaniline (15g, 111mmol.) was placed in a round-bottomed flask and acetic anhydride was added (50mL, 530mmol.). This was refluxed on an oil bath overnight. The following day the excess acetic anhydride was removed under reduced pressure and the resulting solid was recrystallised from methanol to give *N*-acetyl 2-aminonitrobenzene as yellow crystals (14.0g, 70%).

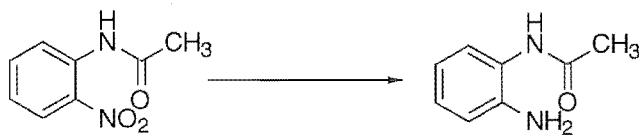
m.p. 90-91°C.

1H NMR ($CDCl_3$) δ 2.18 (s, 1H, CH_3), 7.32 (t, 1H, $J=7.8Hz$, aryl CH), 7.66 (t, 1H, $J=7.8Hz$), 8.01 (d, 1H, $J=7.8Hz$, aryl CH), 8.06 (d, 1H, $J=7.8Hz$, aryl CH).

m/z (EI) 180 (M^+), 138 ($M-CH_3CO^+$), 92.

High Res. $C_8H_8NO_3$ m/z calc. 180.0535; found 180.0536.

Preparation of *N*-acetyl 2-aminoaniline



N-Acetyl 2-aminonitrobenzene (5g, 27.8mmol.) was placed in a round bottomed flask and dissolved in the minimum amount of methanol. 5% Palladium on carbon (3g) was then added and the flask attached via a tap to a hydrogen filled balloon. With the tap closed the air in the reaction vessel was evacuated under the reduced pressure of a water pump, also connected via a tap. The tap to the water pump was then closed and the tap to the hydrogen balloon opened. The reaction was stirred and monitored by TLC, with the hydrogen balloon being refilled where necessary. Once the reaction was complete, the reaction mixture was filtered and the solvent removed under reduced pressure to give *N*-acetyl 2-aminoaniline as a white solid (2.93g, 70%).

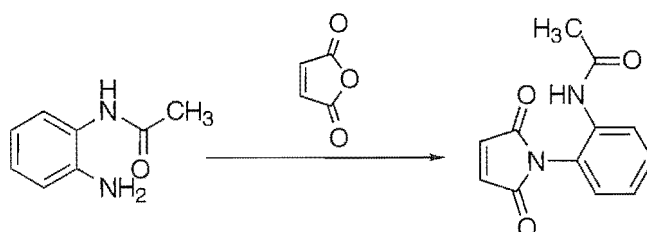
m.p. 110-112°C.

^1H NMR (CDCl_3) δ 2.13 (s, 3H, CH_3), 6.68 (t, 1H, $J=7.8\text{Hz}$, aryl CH), 6.82 (d, 1H, $J=7.8\text{Hz}$, aryl CH), 6.95-7.10 (m, 2H, aryl CH).

m/z (EI) 150 (M^+), 132, 108 ($\text{M}-\text{COCH}_2^+$).

High Res. $\text{C}_8\text{H}_{10}\text{N}_2\text{O}$ m/z calc. 150.07931; found 150.07906.

Preparation of *N*-(2-acetylaminophenyl)maleimide 3.14



N-Acetyl 2-aminonitroaniline (2.93g, 0.195mol.) was dissolved in THF. Maleic anhydride (1.91g, 0.195mol.) in THF was then added to the solution and it was stirred overnight to give a yellow precipitate. To this precipitate sodium acetate (1.0g, 0.151mol.), and acetic anhydride (10mL, 0.106mol.) were added. The flask was heated on a steam bath until completely dissolved and then for a further 30 minutes. The resulting solution was extracted with ethyl acetate. The organic

fractions were combined, dried (MgSO_4), and the solvent concentrated under reduced pressure. The solution was placed in a freezer overnight resulting in crystallisation of a white solid which was recrystallised from ethyl acetate to give *N*-(2-acetylaminophenyl)maleimide as fluffy off-white crystals (2.54g, 60%).

m.p. 180-183°C.

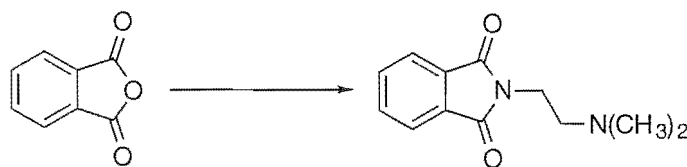
Analysis $\text{C}_{12}\text{H}_{10}\text{N}_2\text{O}_3$ calc. C, 62.60; H, 4.38; N, 12.17; found C, 62.33; H, 4.27; N, 12.21.

^1H NMR (CDCl_3) δ 2.09 (s, 3H, CH_3), 6.91 (s, 2H, COCH), 7.19-7.31 (m, 2H, aryl CH), 7.44 (t, 1H, $J=7.3\text{Hz}$, aryl CH), 7.84 (d, 1H, $J=8.3\text{Hz}$, aryl CH).

m/z (EI) 230.1 (M^+), 188.1 ($\text{M}-\text{COCH}_2^+$), 170.

High Res. $\text{C}_{12}\text{H}_{10}\text{N}_2\text{O}_3$ m/z calc. 230.06914; found 230.06925.

Preparation of *N*-(*N*-dimethylaminoethyl)phthalimide 3.15



A solution of phthalic anhydride (2.0g, 13.5mmol.) in dry toluene (12mL) was brought to reflux. *N,N*-dimethylethylene diamine (1.63mL, 13.5mmol.) in toluene (8mL) was then added and the refluxing continued for two hours. The hot solution was filtered through anhydrous sodium sulfate and the solvent removed under reduced pressure. The residue was then taken up in ethyl acetate and partitioned with 10% sodium bicarbonate solution. The ethyl acetate fractions were combined, dried (MgSO_4), and the ethyl acetate removed under reduced pressure. The resultant solid was recrystallised from toluene and petroleum ether to give *N*-(*N*-dimethylaminoethyl)phthalimide as white crystals (2.7g, 90%).

m.p. 93-95°C.

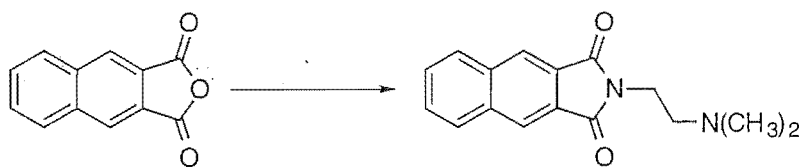
^1H NMR (CDCl_3) δ 2.29 (s, 6H, CH_3), 2.60 (t, 2H, $J=6.8\text{Hz}$, $\text{CONCH}_2\text{CH}_2$), 3.81 (t, 2H, $J=6.8\text{Hz}$, CONCH_2), 7.71 (m, 2H, aryl CH), 7.84 (m, 2H, aryl CH).

m/z (EI) 218.1 (M^+), 58.1.

High Res. $\text{C}_{12}\text{H}_{14}\text{N}_2\text{O}_2$ m/z calc. 218.10553, found 218.10509.

IR ν_{max} (Nujol) 2772, 2360, 1767, 1707, 1460, 1398, 1377, 1320, 1111, 1016, 936, 721 cm^{-1} .

Preparation of *N*-(*N*-dimethylaminoethyl)naphthalimide 3.22



A solution of 2,3-naphthalic anhydride **2.19** (2g, 10.1mmol.) in dry toluene (12mL) was brought to reflux. *N,N*-dimethylethylene diamine (1.22mL, 10.1mmol.) in toluene (8mL) was then added and the refluxing continued for two hours. The hot solution was filtered through anhydrous sodium sulfate and the solvent removed under reduced pressure. The residue was then taken up in ethyl acetate and partitioned with 10% sodium bicarbonate solution. The ethyl acetate fractions were combined, dried (MgSO_4), and the ethyl acetate removed under reduced pressure. Recrystallisation from toluene and petroleum ether gave *N*-(*N*-dimethylaminoethyl)naphthalimide as long white needles (2.3g, 85%).

m.p. 128-130°C.

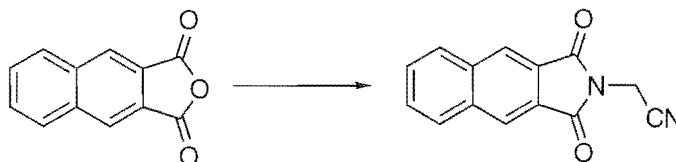
^1H NMR (CDCl_3) δ 2.27 (s, 6H, CH_3), 2.65 (t, 2H, $J=6.8\text{Hz}$, $\text{CONCH}_2\text{CH}_2$), 3.88 (t, 2H, $J=6.8\text{Hz}$, CONCH_2), 7.69 (m, 2H, aryl CH), 8.06 (m, 2H, aryl CH), 8.33 (s, 2H, COCCH).

m/z (EI) 268 (M^+), 210, 154, 126, 71.

High Res. $\text{C}_{16}\text{H}_{16}\text{N}_2\text{O}_2$ calc. 268.1212, found 268.1214.

IR ν_{max} (Nujol) 1708, 1460, 1377, 1151, 1118, 767 cm^{-1} .

Preparation of *N*-(2-cyanomethyl)naphthalimide 3.23



A solution of 2,3-naphthalic anhydride **2.19** (200mg, 1.01mmol.) in dry toluene (5mL) was brought to reflux. Aminoacetonitrile (93.4mg, 1.01mmol.) in toluene (2.5mL) was then added and the refluxing continued for two hours. The hot solution was filtered through anhydrous sodium sulfate and the solvent removed under reduced pressure. The residue was then taken up in ethyl acetate and partitioned with 10% sodium bicarbonate solution. The ethyl acetate fractions were combined, dried (MgSO_4), and the ethyl acetate removed under reduced pressure. The resultant solid was recrystallised from toluene and petroleum ether to give *N*-(2-cyanomethyl)naphthalimide as pale brown crystals (50mg, 21%).

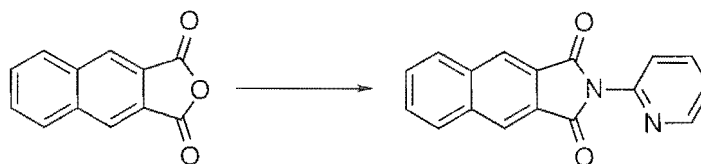
m.p. 197-198°C.

^1H NMR (CDCl_3) δ 4.65 (s, 2H, CH_2), 7.83 (m, 2H, aryl CH), 8.16 (m, 2H, aryl CH), 8.56 (s, 2H, COCH).

m/z (EI) 236 (M^+), 192, 154, 126

High Res. $\text{C}_{14}\text{H}_8\text{N}_2\text{O}_2$ m/z calc. 236.0586, found 236.0585.

Preparation of *N*-(2-pyridyl)naphthalimide 3.24



A solution of 2,3-naphthalic anhydride **2.19** (200mg, 1.01mmol.) in dry toluene (5mL) was brought to reflux. 2-Amino pyridine (95mg, 1.01mmol.) in toluene (2.5mL) was then added and the refluxing continued for two hours. The hot solution was filtered through anhydrous sodium sulfate and the solvent removed under reduced pressure. The residue was then taken up in ethyl acetate and partitioned with 10% sodium bicarbonate solution. The ethyl acetate fractions were combined, dried (MgSO_4), and the ethyl acetate removed under reduced pressure. The resultant solid was recrystallised from toluene and petroleum ether to give *N*-(2-pyridyl)naphthalimide as pale brown crystals (55mg, 20%).

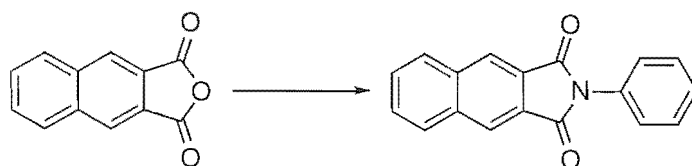
m.p. 190°C (decomp.).

^1H NMR (CDCl_3) δ 7.40 (m, 1H, aryl CH), 7.52 (d, 1H, $J=7.8\text{Hz}$, aryl CH), 7.74 (m, 2H, aryl CH), 7.94 (m, 1H, aryl CH), 8.12 (m, 2H, aryl CH), 8.50 (s, 2H, aryl CH), 8.74 (m, 1H, aryl CH).

m/z (EI) 274 (M^+), 246, 126.

High Res. $\text{C}_{17}\text{H}_{10}\text{N}_2\text{O}_2$ m/z calc. 274.0742, found 274.0741.

Preparation of *N*-(phenyl)naphthalimide **3.25**



A solution of 2,3-naphthalic anhydride **2.19** (500mg, 2.52mmol.) in dry toluene (10mL) was brought to reflux. Aniline (0.5mL, 5.49mmol.) in toluene (2.5mL) was then added and the refluxing continued for two hours. The solvent removed under reduced pressure. The residue was then taken up in ethyl acetate and partitioned with 10% sodium bicarbonate solution. The ethyl acetate fractions were combined, dried (MgSO_4), and the ethyl acetate removed under reduced

pressure. The resultant solid was recrystallised from toluene and petroleum ether to give *N*-(phenyl)naphthalimide as pale brown crystals (237mg, 34%).

m.p. 279-282°C.

^1H NMR (CDCl_3) δ 7.43-7.54 (m, 5H, aryl CH), 7.74 (m, 2H, aryl CH), 8.12 (m, 2H, aryl CH), 8.47 (s, 2H, aryl CH).

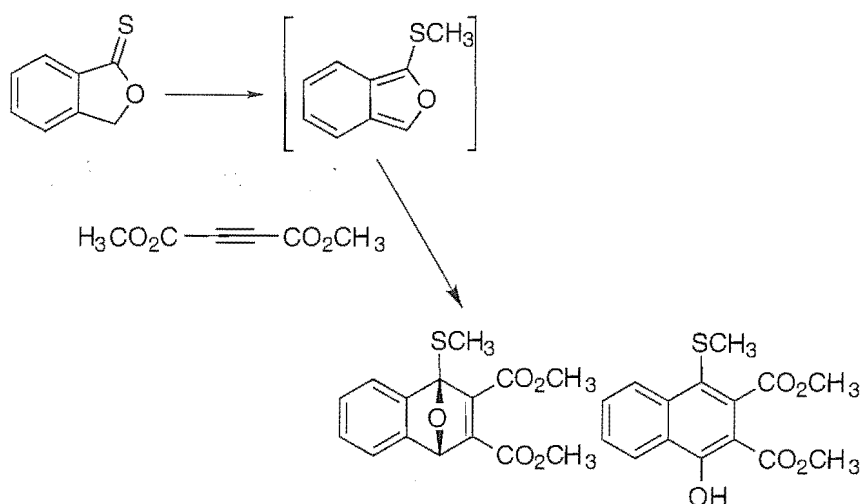
m/z (EI) 273.1 (M^+), 229.1, 126.0.

High Res. $\text{C}_{18}\text{H}_{11}\text{NO}_2$ m/z calc. 273.07898, found 273.07874.

IR ν_{max} (CDCl_3) 1774.4, 1716.5, 1500.5, 1375.2 cm^{-1} .

7.4 Chapter 4 Experimental

Preparation of dimethyl 1-methylsulfanyl-11-oxatricyclo[6.2.1.0^{2,7}]undeca-2,4,6,9-tetraene-9,10-dicarboxylate 4.17 and dimethyl 1-hydroxy-4-(methylsulfanyl)naphthalene-2,3-dicarboxylate 4.18⁶⁴



n-Butyl lithium (0.88mL of a 1.0M solution in hexane, 0.88mmol.) was added dropwise to a stirred solution of diisopropylamine (0.12mL, 0.88mmol.) in THF at 0°C. The solution was stirred for ten minutes at 0°C and then cooled to -78°C. A solution of 1(3H)-isobenzofuranthione (116mg, 0.77mmol.) in THF was added and the mixture was stirred for 1 hour at -78°C. Methyl iodide (0.055mL, 0.88mmol.) was added dropwise to the solution, which was then allowed to warm to room temperature for 2 hours. Dimethyl acetylenedicarboxylate (0.105mL, 0.85mmol.) in THF was added and the reaction stirred overnight. The solvent was removed under reduced pressure to give an oil which was partitioned between ethyl acetate and water. The organic layer was dried (MgSO_4) and the solvent removed under reduced pressure. Purification by radial silica chromatography gave two

products; dimethyl 1-methylsulfanyl-11-oxatricyclo[6.2.1.0^{2,7}]undeca-2,4,6,9-tetraene-9,10-dicarboxylate **4.17** as a pale yellow oil (67.3mg, 25%)

¹H NMR (CDCl₃) δ 2.31 (s, 3H, SCH₃), 3.77 (s, 3H, OCH₃), 3.84 (s, 3H, OCH₃), 5.97 (s, 1H, OCH), 7.11 (m, 2H, aryl CH), 7.39 (m, 2H, aryl CH).

m/z (EI) 306 (M⁺), 164 (M-C₆H₆O₄⁺).

High Res. C₁₅H₁₄SO₅ *m/z* calc. 306.0562; found 306.0572.

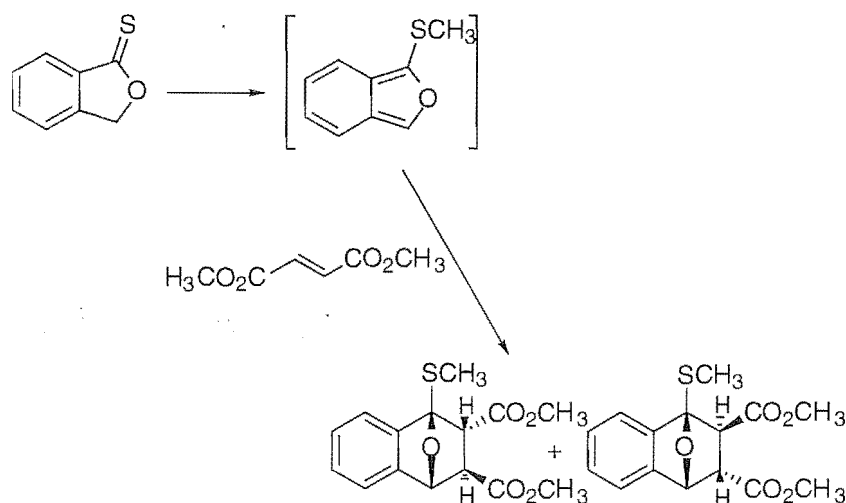
and dimethyl 1-hydroxy-4-(methylsulfanyl)naphthalene-2,3-dicarboxylate **4.18** as a yellow oil (26.9mg, 10%).

¹H NMR (CDCl₃) δ 2.31 (s, 3H, SCH₃), 3.99 (s, 6H, CO₂CH₃), 7.63 (t, 1H, J=8.3Hz, aryl CH), 7.79 (t, 1H, J=8.3Hz, aryl CH), 8.50 (d, 1H, J=8.3Hz, aryl CH), 8.60 (d, 1H, J=8.3Hz, aryl CH).

m/z (EI) 306 (M⁺), 290 (M-O⁺), 276 (M-CH₂O⁺), 259, 227, 217, 201, 171

High Res. C₁₅H₁₄SO₅ *m/z* calc. 306.0562; found 306.0556.

Preparation of dimethyl 9-*exo*-10-*endo*-1-methylsulfanyl-11-oxatricyclo[6.2.1.0^{2,7}]undeca-2,4,6-triene-9,10-dicarboxylate **4.19**
 and dimethyl 9-*endo*-10-*exo*-1-methylsulfanyl-11-oxatricyclo[6.2.1.0^{2,7}]undeca-2,4,6-triene-9,10-dicarboxylate **4.20**⁶⁴



n-Butyl lithium (0.48mL of a 1.0M solution in hexane, 0.48mmol.) was added dropwise to a stirred solution of diisopropylamine (0.067mL, 0.48mmol.) in THF at 0°C. The solution was stirred for ten minutes at 0°C and then cooled to -78°C. A solution of 1(3H)-isobenzofuranthione (60mg, 0.40mmol.) in THF was added and the mixture was stirred for 1 hour at -78°C. Methyl iodide (0.029mL, 0.48mmol.) was added dropwise to the solution, which was then allowed to warm to room temperature for 2 hours. Dimethyl fumarate (69mg, 0.48mmol.) in THF was added and the reaction stirred overnight. The solvent was removed under reduced pressure to give an oil which was partitioned between ethyl acetate and water. The organic layer was dried (MgSO₄) and the solvent removed under reduced pressure. This was purified by radial silica chromatography to give a mixture of dimethyl 9-*exo*-10-*endo*-1-methylsulfanyl-11-oxatricyclo[6.2.1.0^{2,7}]undeca-2,4,6-triene-9,10-dicarboxylate **4.19** and dimethyl 9-*endo*-10-*exo*-1-methylsulfanyl-11-

oxatricyclo[6.2.1.0^{2,7}]undeca-2,4,6-triene-9,10-dicarboxylate **4.20** together as a pale yellow oil (70mg, 57%).

4.19 (major) ¹H NMR (CDCl₃) δ 2.22 (s, 3H, SCH₃), 3.21 (d, 1H, J=4.4Hz, COCH), 3.55 (s, 3H, CO₂CH₃), 3.79 (d, 1H, J=4.4Hz, COCH), 3.80 (s, 3H, CO₂CH₃), 5.68 (m, 1H, OCH), 7.27 (m, 3H, aryl CH), 7.37 (d, 1H, J=7.8Hz, aryl CH).

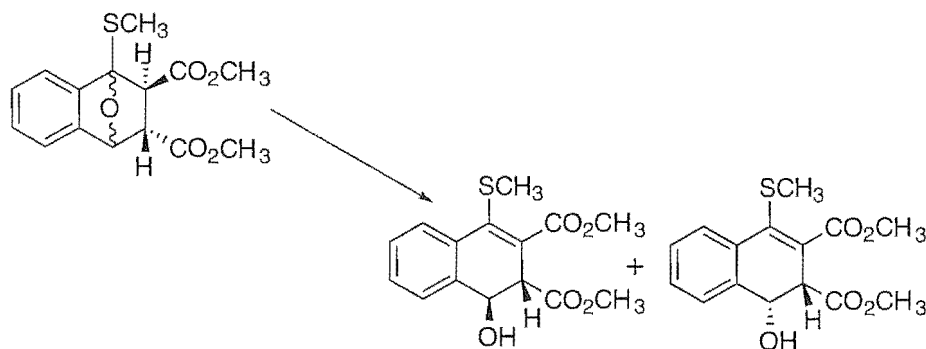
4.20 (minor) ¹H NMR (CDCl₃) δ 2.09 (s, 3H, SCH₃), 3.10 (d, 1H, J=4.4Hz, COCH), 3.53 (s, 3H, CO₂CH₃), 3.83 (s, 3H, CO₂CH₃), 4.15 (d, 1H, J=4.4Hz, COCH), 5.67 (s, 1H, OCH), 7.27 (m, 3H, aryl CH), 7.37 (d, 1H, J=7.8Hz, aryl CH).

¹³C NMR (CDCl₃) 12.25, 52.19, 52.23, 52.37, 52.63, 52.67, 52.84, 80.60, 119.73, 120.70, 127.52, 127.75, 128.06, 128.23, 169.39.

m/z (EI) 308 (M⁺), 164 (M-C₆H₆O₄⁺).

High Res. C₁₅H₁₆SO₅ *m/z* calc. 308.0718; found 308.0727.

Preparation of 1-*exo*-1-hydroxy-4-methylsulfanyl-1,2-dihydronaphthalene-2,3-dicarboxylate **4.21a and 1-*endo*-1-hydroxy-4-methylsulfanyl-1,2-dihydronaphthalene-2,3-dicarboxylate **4.21b****



Dimethyl 1-methylsulfanyl-11-oxatricyclo[6.2.1.0^{2,7}]undeca-2,4,6-triene-9,10-dicarboxylate (37mg, 0.120mmol.) was stirred in dichloromethane while trifluoroacetic acid (14mg, 10mL, 0.240mmol.) in dichloromethane was added

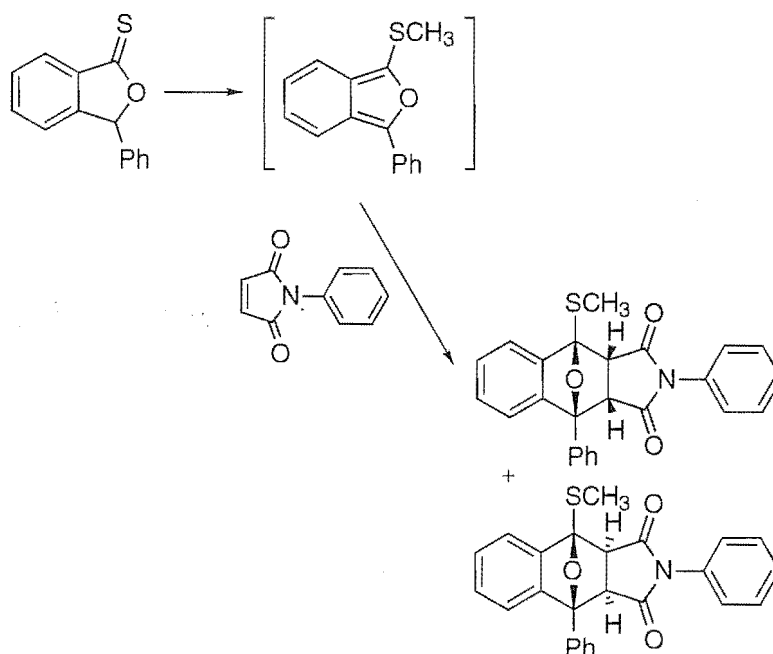
dropwise. The solution turned yellow straight away. The solution was stirred overnight. The dichloromethane was removed under reduced pressure to give 1-*exo*-1-hydroxy-4-methylsulfanyl-1,2-dihydronaphthalene-2,3-dicarboxylate **4.21a** and 1-*endo*-1-hydroxy-4-methylsulfanyl-1,2-dihydronaphthalene-2,3-dicarboxylate **4.21b** together as a pale yellow oil (30mg, 82%).

4.21a (major) ^1H NMR (CDCl_3) δ 2.20 (s, 3H, SCH₃), 3.54 (s, 3H, CO₂CH₃), 3.89 (s, 3H, CO₂CH₃), 4.15 (d, 1H, J=5.8Hz, COCH), 5.12 (d, 1H, J=5.9Hz, C(OH)H), 7.34-7.46 (m, 2H, aryl CH), 7.64 (d, 1H, J=6.9Hz, aryl CH), 7.75 (d, 1H, J=7.3Hz, aryl CH).

4.21b (minor) ^1H NMR (CDCl_3) δ 2.24 (s, 3H, SCH₃), 3.59 (s, 3H, CO₂CH₃), 3.88 (s, 3H, CO₂CH₃), 4.19 (d, 1H, J=4.4Hz, COCH), 5.17 (d, 1H, J=4.4Hz, C(OH)H), 7.34-7.46 (m, 2H, aryl CH), 7.63 (d, 1H, J=6.9Hz, aryl CH), 7.90 (d, 1H, J=7.3Hz, aryl CH).

m/z (EI) 308 (M^+), 290 ($\text{M}-\text{H}_2\text{O}^+$).

Preparation of 9-endo-13-endo-1-methylsulfanyl-8,11-diphenyl-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione 4.26 and 9-exo-13-exo-1-methylsulfanyl-8,11-diphenyl-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione 4.29⁶⁷



n-Butyl lithium (2.5mL of a 1.6M solution in hexane, 4.00mmol.) was added dropwise to a stirred solution of diisopropylamine (0.56mL, 4.00mmol.) in THF at 0°C. The solution was stirred for ten minutes at 0°C and then cooled to -78°C. A solution of 3-phenyl-1(3H)-isobenzofuranthione (750mg, 3.33mmol.) in THF was added and the mixture was stirred for 1 hour at -78°C. Methyl iodide (0.25mL, 4.00mmol.) was added dropwise to the solution, which was then allowed to warm to room temperature over a period of 2 hours. *N*-Phenyl maleimide (634mg, 3.66mmol.) in THF was added and the reaction was stirred overnight. The solvent was removed under reduced pressure to give a brown/red oil which was partitioned between ethyl acetate and water. The organic layer was dried (MgSO₄) and the solvent removed under reduced pressure. Purification by radial silica

chromatography and mixed solvent recrystallisation from ethyl acetate and petroleum ether gave 9-*endo*-13-*endo*-1-methylsulfanyl-8,11-diphenyl-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione **4.26** as white crystals (660mg, 48%)

m.p. 203-205°C (lit. 202-205°C).⁶⁷

¹H NMR (CDCl₃) δ 2.33 (s, 3H, SCH₃), 3.91 (d, 1H, J=8.3Hz, COCH), 4.13 (d, 1H, J=8.3Hz, COCH), 6.46 (m, 2H, aryl CH), 7.07 (d, 1H, J=7.3Hz, aryl CH), 7.21-7.51 (m, 9H, aryl CH), 7.95 (m, 2H, aryl CH).

¹³C NMR (CDCl₃) δ 12.49, 54.15, 54.48, 90.51, 94.61, 121.25, 121.47, 126.27, 127.01, 128.50, 128.61, 128.70, 128.78, 128.89, 130.94, 135.84, 139.96, 144.68, 171.52, 172.96.

m/z (EI) 413 (M⁺), 365 (M-SCH₃⁺), 240, 173.

High Res C₂₅H₁₉NSO₃ *m/z* calc. 413.1086; found 413.1086.

IR *v*_{max} (CDCl₃) 1755, 1700, 1562, 1500, 1381 cm⁻¹.

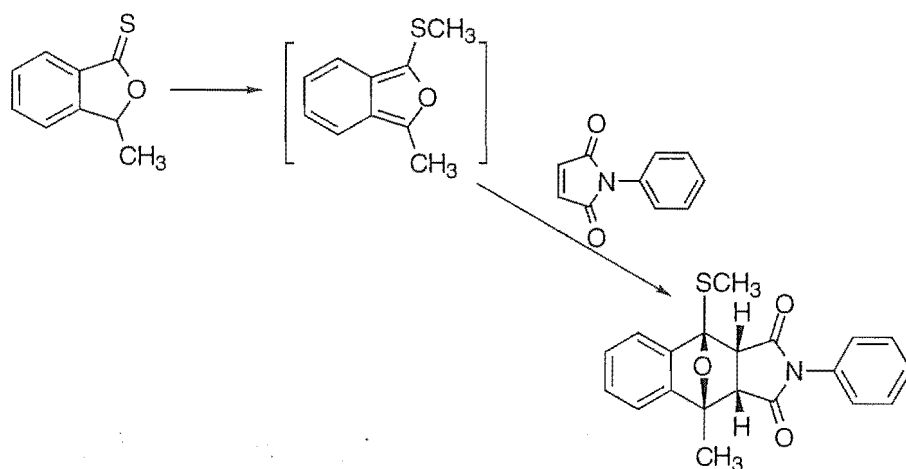
and 9-*exo*-13-*exo*-1-methylsulfanyl-8,11-diphenyl-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione **4.29** as white crystals (14mg, 1%).

m.p. 202-203°C.

¹H NMR (CDCl₃) δ 2.25 (s, 3H, SCH₃), 3.34 (d, 1H, J=6.8Hz, COCH), 3.59 (d, 1H, J=6.9Hz, COCH), 7.23-7.51 (m, 13H, aryl CH), 7.67 (d, 1H, J=7.3Hz, aryl CH).

m/z (EI) 413 (M⁺), 365 (M-SCH₃⁺), 240, 173.

Preparation of 9-endo-13-endo-1-methylsulfanyl-8-methyl-11-phenyl-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione 4.27



n-Butyl lithium (1.41mL of a 1.6M solution in hexane, 2.26mmol.) was added dropwise to a stirred solution of diisopropylamine (0.31mL, 2.26mmol.) in THF at 0°C. The solution was stirred for ten minutes at 0°C and then cooled to -78°C. A solution of 3-methyl-1(3H)-isobenzofuranthione (309mg, 1.88mmol.) in THF was added and the mixture was stirred for 1 hour at -78°C. Methyl iodide (0.14mL, 2.26mmol.) was added dropwise to the solution, which was allowed to warm to room temperature for 2 hours. *N*-Phenyl maleimide (326mg, 1.88mmol.) in THF was added and the reaction stirred overnight. The solvent was removed under reduced pressure to give an oil which was partitioned between ethyl acetate and water. The organic layer was dried (MgSO₄) and the solvent removed under reduced pressure. Radial silica chromatography followed by mixed-solvent recrystallisation from ethyl acetate and petroleum ether gave 9-endo-13-endo-1-methylsulfanyl-8-methyl-11-phenyl-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione as fine white needles (245mg, 37%).

m.p. 196-197°C.

Analysis $C_{20}H_{17}NSO_3$ calc. C, 68.36; H, 4.87; N, 3.99; found C, 68.37; H, 4.75; N, 3.99.

1H NMR ($CDCl_3$) δ 2.08 (s, 3H, CH_3), 2.27 (s, 3H, SCH_3), 3.67 (d, 1H, $J=8.3Hz$, $COCH$), 3.82 (d, 1H, $J=8.3Hz$, $COCH$), 6.41 (m, 2H, aryl CH), 7.23-7.38 (m, 7H, aryl CH).

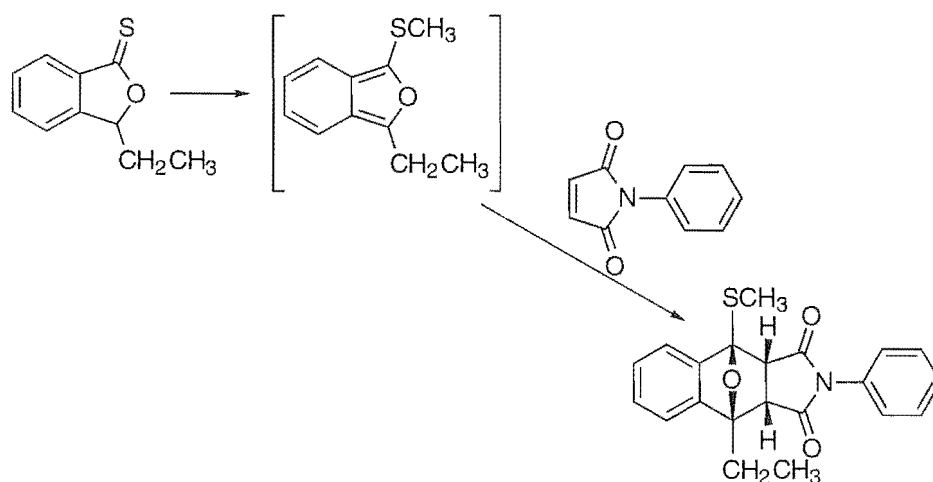
^{13}C NMR ($CDCl_3$) δ 172.75, 171.65, 143.77, 140.52, 130.81, 128.75, 128.67, 128.51, 128.31, 126.30, 126.14, 125.98, 121.36, 120.27, 94.80, 86.83, 55.04, 54.25, 17.44, 12.32.

m/z (FAB) 351 (M^+), 333, 300, 178, 173, 128, 91.

High Res. $C_{20}H_{17}NSO_3$ calc. 351.09292; found 351.09228.

IR ν_{max} (Nujol) 3032, 3020, 1717, 1499, 1460, 1385, 1279, 1182, 1117, 1034, 908, 853, 799, 764 cm^{-1} .

Preparation of 9-endo-13-endo-1-methylsulfanyl-8-ethyl-11-phenyl-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione 4.28



n-Butyl lithium (2.39mL of a 1.6M solution in hexane, 3.84mmol.) was added dropwise to a stirred solution of diisopropylamine (0.54mL, 3.84mmol.) in THF at 0°C. The solution was stirred for ten minutes at 0°C and then cooled to -78°C using

a dry ice and acetone bath. A solution of 3-ethyl-1(3H)-isobenzofuranthione (570mg, 3.20mmol.) in THF was added and the mixture was stirred for 1 hour at -78°C. Methyl iodide (0.14mL, 3.84mmol.) was added dropwise to the solution, which was then allowed to warm to room temperature over a period of 2 hours. *N*-Phenyl maleimide (550mg, 3.20mmol.) in THF was added and the reaction was stirred overnight. The solvent was removed under reduced pressure to give an oil. This was partitioned between ethyl acetate and water. The organic layer was dried (MgSO₄) and the solvent removed under reduced pressure. This was purified by radial chromatography followed by mixed solvent recrystallisation from ethyl acetate and petroleum ether to give 9-*endo*-13-*endo*-1-methylsulfanyl-8-ethyl-11-phenyl-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione as white crystals (55mg, 5%).

m.p. 205-207°C.

Analysis C₂₁H₁₉NSO₃ calc. C, 69.02; H, 5.24; N, 3.83; found C, 69.10; H, 5.18; N, 3.79.

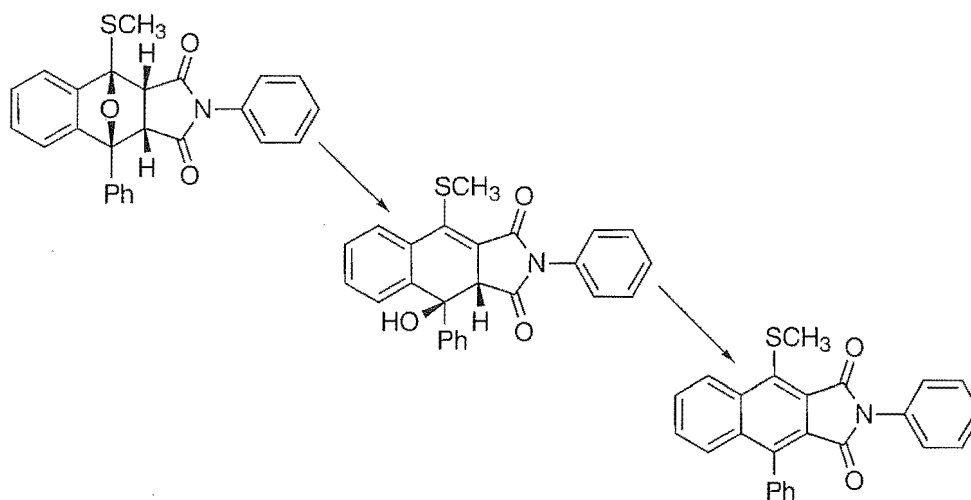
¹H NMR (CDCl₃) δ 1.20 (t, 3H, J=7.8Hz, CH₃), 2.27 (s, 3H, SCH₃), 2.39-2.65 (m, 2H, CH₂), 3.72 (d, 1H, J=8.3Hz, COCH), 3.79 (d, 1H, J=8.3Hz, COCH), 6.42 (m, 2H, aryl CH), 7.23-7.40 (m, 7H, aryl CH).

m/z (EI) 365 (M⁺), 347, 337, 318, 217, 199.

High Res. C₂₁H₁₉NSO₃ *m/z* calc. 365.10857; found 365.10821.

IR ν_{\max} 3028.0, 3014.5, 1716.5, 1498.6, 1379.0, 1230.5, 1222.8, 1211.2, 1199.6, 1182.3 cm⁻¹.

Preparation of *N*-phenyl-1-methylsulfanyl-4-phenyl-naphthalene-2,3-dicarboximide 4.32



9-*endo*-13-*endo*-1-Methylsulfanyl-8,11-diphenyl-11-aza-14-oxatetracyclo [6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione (90mg, 0.218mmol.) was stirred in dichloromethane while trifluoroacetic acid (25mg, 17mL, 0.218mmol.) in dichloromethane was added dropwise. A pale yellow colour was observed to form rapidly. The solution was stirred overnight. The dichloromethane was then removed under reduced pressure to give the intermediate product *N*-phenyl-1-methylsulfanyl-4-hydroxy-4-phenyl-3,4-dihydronaphthalene-2,3-dicarboximide as an oil.

¹H NMR (CDCl₃) δ 2.63 (s, 3H, SCH₃), 4.22 (s, 1H, COCH), 6.98 (m, 2H, aryl CH), 7.18-7.26 (m, 5H, aryl CH), 7.36-7.59 (m, 5H, aryl CH), 7.78 (d, 1H, J=7.8Hz, aryl CH), 8.15 (d, 1H, J=7.8Hz, aryl CH).

m/z (EI) 395 (M-H₂O⁺), 362, 303.

Upon a further attempt to purify *N*-phenyl-1-methylsulfanyl-4-hydroxy-4-phenyl-3,4-dihydronaphthalene-2,3-dicarboximide by thin layer chromatography it was found to have dehydrated on the silica. This product was recrystallised from ethyl acetate and petroleum ether to give *N*-phenyl-1-methylsulfanyl-4-phenyl-naphthalene-2,3-dicarboximide as off-white crystals (50mg, 58%).

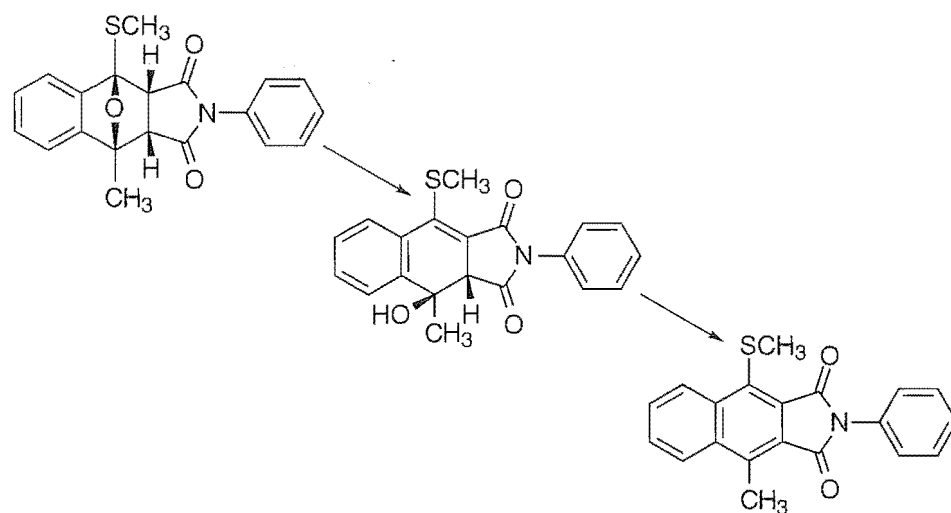
m.p. 200-201°C.

^1H NMR (CDCl_3) δ 2.71 (s, 3H, SCH_3), 7.36-7.46 (m, 7H, aryl CH), 7.53-7.55 (m, 3H, aryl CH), 7.66 (d, 1H, $J=8.3\text{Hz}$, aryl CH), 7.81 (m, 2H, aryl CH), 9.10 (d, 1H, $J=7.8\text{Hz}$, aryl CH).

m/z (EI) 395 (M^+), 362, 303.

High Res. $\text{C}_{25}\text{H}_{17}\text{NSO}_2$ m/z calc. 395.0979, found 395.0973.

Preparation of *N*-phenyl-1-methylsulfanyl-4-methyl-naphthalene-2,3-dicarboximide 4.33



9-*endo*-13-*endo*-1-Methylsulfanyl-8-methyl-11-phenyl-11-aza-14-oxatetracyclo [6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione (42mg, 0.195mmol.) was dissolved in dichloromethane and trifluoroacetic acid (0.01mL, 0.215mmol.) added, resulting in an instant colour change of the solution to yellow. The solution was then stirred for 3 hours after which the solvent and excess trifluoroacetic acid was removed under reduced pressure, to give the intermediate product *N*-phenyl-1-methylsulfanyl-4-hydroxy-4-methyl-3,4-dihydronaphthalene-2,3-dicarboximide as an oil (33mg, 48%).

^1H NMR (CDCl_3) δ 1.45 (s, 3H, CH_3), 2.55 (s, 3H, SCH_3), 3.92 (s, 1H, COCH), 7.34 (d, 2H, $J=8.8\text{Hz}$, aryl CH), 7.43-7.54 (m, 5H, aryl CH), 7.80 (d, 1H, $J=7.9\text{Hz}$, aryl CH), 8.03 (d, 1H, $J=7.8\text{Hz}$, aryl CH).

To complete the aromatisation the *N*-phenyl-1-methylsulfanyl-4-hydroxy-4-methyl-3,4-dihydronaphthalene-2,3-dicarboximide was dissolved in acetonitrile and concentrated hydrochloric acid added dropwise until a pH of 1 was reached. This solution was then stirred for 5 days and monitored by TLC, after which the solvent was removed under reduced pressure to give a solid. This was purified by a mixed solvent recrystallisation from ethyl acetate and petroleum ether to give *N*-phenyl-1-methylsulfanyl-4-methyl-naphthalene-2,3-dicarboximide as pale yellow crystals (18mg, 2 steps 28%).

m.p. 138-140°C.

Analysis $\text{C}_{20}\text{H}_{15}\text{NO}_2\text{S}$ calc. C, 72.05; H, 4.53; N, 4.20; found C, 71.98; H, 4.32; N, 4.15.

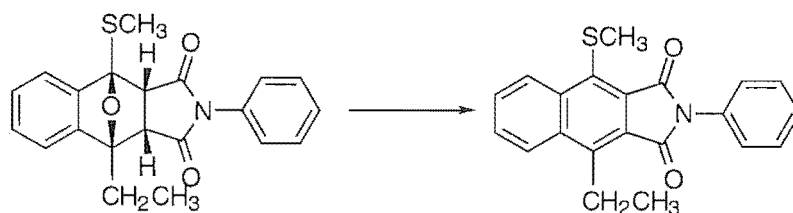
^1H NMR (CDCl_3) δ 2.62 (s, 3H, CH_3), 3.17 (s, 3H, SCH_3), 7.43-7.56 (m, 5H, aryl CH), 7.80 (m, 2H, aryl CH), 8.30 (m, 1H, aryl CH), 9.05 (m, 1H aryl CH).

m/z (EI) 333.1 (M^+), 300.1.

High Res. $\text{C}_{20}\text{H}_{15}\text{NO}_2\text{S}$ calc. 333.08235; found 333.08198.

IR ν_{max} 1764.7, 1712.7, 1498.6, 1382.9, 1211.2, 1193.9, 1134.1 cm^{-1} .

Preparation of *N*-phenyl-1-methylsulfanyl-4-ethyl-naphthalene-2,3-dicarboximide 4.34



9-*endo*-13-*endo*-1-Methylsulfanyl-8-ethyl-11-phenyl-11-aza-14-oxatetracyclo [6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione (6mg, 0.0164mmol.) was dissolved in acetonitrile and concentrated hydrochloric acid added dropwise until a pH of 1 was reached. This solution was then stirred for 5 days and monitored by TLC, after which the solvent was removed under reduced pressure giving a clear oil. This was purified by radial silica chromatography to give *N*-phenyl-1-methylsulfanyl-4-ethyl-naphthalene-2,3-dicarboximide as an off-white solid (4mg, 70%).

m.p. 180-181°C.

Analysis C₂₁H₁₇NSO₂ calc. C, 72.60%; H, 4.93%; N, 4.03%; found C, 72.81%; H, 4.95%; N, 4.00%.

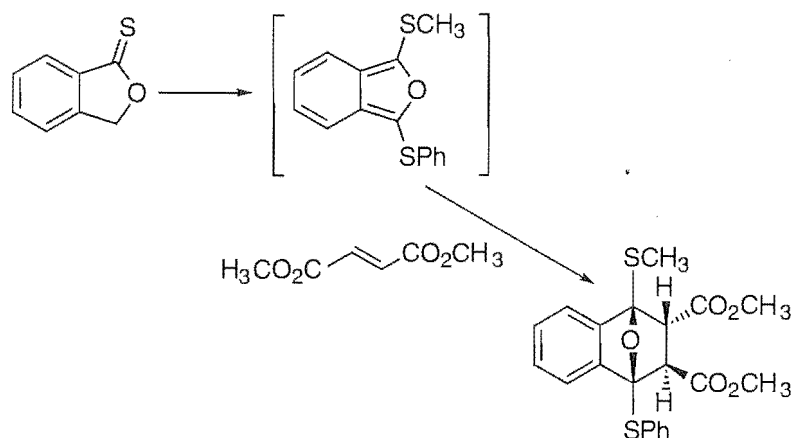
¹H NMR (CDCl₃) δ 1.40 (t, 3H, J=7.8Hz, CH₃), 2.62 (s, 3H, SCH₃), 3.74 (q, 2H, J=7.3Hz, CH₂), 7.42-7.54 (m, 5H, aryl CH), 7.80 (m, 2H, aryl CH), 8.35 (m, 1H, aryl CH), 9.09 (m, 1H, aryl CH).

m/z (EI) 347 (M⁺, 100%), 314, 254, 151, 91.

High Res. C₁₂H₁₇NO₂S *m/z* calc. 347.09800; found 347.09837.

IR *ν*_{max} (CDCl₃) 1762.8, 1710.7, 1500.5, 1384.8, 1199.6, 1134.1 cm⁻¹.

Preparation of dimethyl 9-*exo*-10-*endo*-1-methylsulfanyl-8-phenylsulfanyl-11-oxatricyclo[6.2.1.0^{2,7}]-undeca-2,4,6-triene-9,10-dicarboxylate 4.36



n-Butyl lithium (1.42mL of a 1.0M solution in hexane, 1.42mmol.) was added dropwise to a stirred solution of diisopropylamine (0.2mL, 1.42mmol.) in THF at 0°C. The solution was stirred for ten minutes at 0°C and then cooled to -78°C. A solution of thionophthalide (97mg, 0.65mmol.) in THF was added and the mixture was stirred for 1 hour at -78°C. Phenylsulfenyl chloride (0.077mL, 0.70mmol.) was added and the temperature kept at -78°C for a further 15 minutes, after which the solution was warmed to 0°C for 1 hour. Methyl iodide (0.05mL, 0.80mmol.) was added dropwise to the solution, which was then allowed to warm to room temperature for 30 minutes. Dimethyl fumarate (102mg, 0.70mmol.) in THF was added and the reaction was stirred overnight. The solvent was removed under reduced pressure to give an orange oil which was partitioned between ethyl acetate and water. The organic layer was dried (MgSO₄) and the solvent removed under reduced pressure. This was purified twice by radial chromatography to give dimethyl 9-*exo*-10-*endo*-1-methylsulfanyl-8-phenylsulfanyl-11-oxatricyclo[6.2.1.0^{2,7}]-undeca-2,4,6-triene-9,10-dicarboxylate as a yellow oil (1mg, 0.4%).

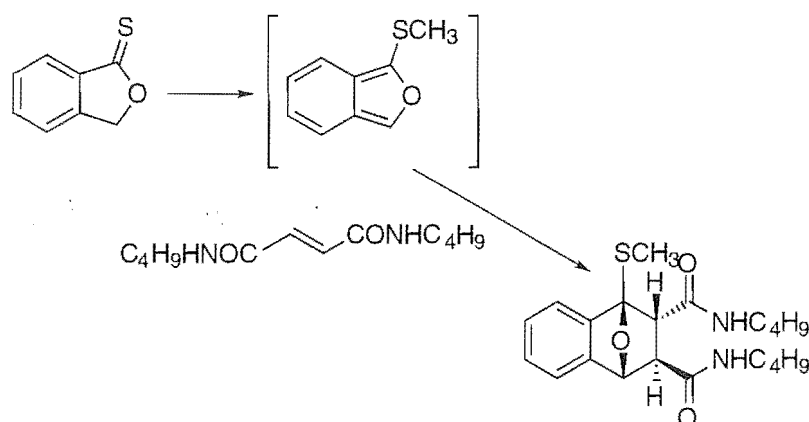
¹H NMR (CDCl₃) δ 2.38 (s, 3H, SCH₃), 3.20 (d, 1H, J=3.9Hz, COCH), 3.36 (d, 1H, J=4.4Hz, COCH), 3.51 (s, 3H, CO₂CH₃), 3.81 (s, 3H, CO₂CH₃),

7.06-7.80 (m, 7H, aryl CH), 7.99 (d, 1H, $J=8.3\text{Hz}$, aryl CH), 8.07 (d, 1H, $J=7.3\text{Hz}$, aryl CH).

m/z (EI) 416 (M^+), 307 ($M-\text{SC}_6\text{H}_5^+$), 272, 257, 149.

High Res. $\text{C}_{21}\text{H}_{20}\text{S}_2\text{O}_5$ m/z calc. 416.07529; found 416.07515.

Preparation of *N,N'*-dibutyl 9-*exo*-10-*endo*-1-methylsulfanyl-11-oxatricyclo[6.2.1.0^{2,7}]undeca-2,4,6-triene-9,10-dicarboxamide 4.44



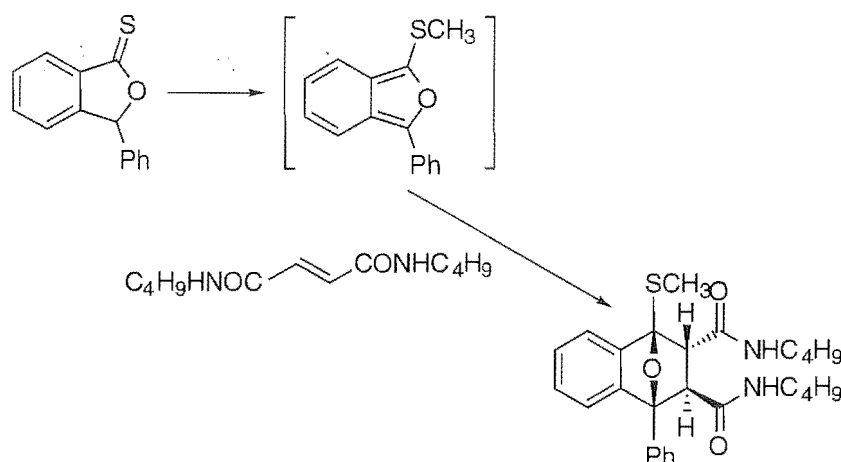
n-Butyl lithium (0.33mL of a 1.6M solution in hexane, 0.510mmol.) was added dropwise to a stirred solution of diisopropylamine (0.072mL, 0.510mmol.) in THF at 0°C. The solution was stirred for ten minutes at 0°C and then cooled to -78°C. A solution of thionophthalide (64.7mg, 0.431mmol.) in THF was added and the mixture was stirred for 1 hour at -78°C. Methyl iodide (0.033mL, 0.510mmol.) was added dropwise to the solution, which was then allowed to warm to room temperature over a period of 2 hours. *N,N'*-Dibutyl fumaramide (101mg, 0.446mmol.) in THF was added and the reaction was stirred overnight. The solvent was removed under reduced pressure to give a red oil. This was partitioned between ethyl acetate and water. The organic layer was dried (MgSO_4) and the solvent removed under reduced pressure to give *N,N'*-dibutyl 9-*exo*-10-*endo*-1-methylsulfanyl-11-oxatricyclo[6.2.1.0^{2,7}]undeca-2,4,6-triene-9,10-dicarboxamide as a clear oil (33.5mg, 20%).

^1H NMR (CDCl_3) δ 0.84 (m, 3H, CH_3), 0.92 (m, 4H, CH_2CH_3), 1.14-1.49 (m, 4H, NHCH_2CH_2), 2.28 (s, 3H, SCH_3), 2.75 (d, 1H, $J=4.9\text{Hz}$, COCH), 2.94 (d, 1H, $J=5.4\text{Hz}$, COCH), 3.03-3.36 (m, 4H, NHCH_2), 5.71 (d, 1H, $J=9.3\text{Hz}$, OCH), 7.36-7.70 (m, 3H, aryl CH), 7.95 (d, 1H, $J=7.3\text{Hz}$, aryl CH).

m/z (EI) 389 (M^+), 316, 200.

High Res. $\text{C}_{21}\text{H}_{29}\text{N}_2\text{SO}_3$ m/z calc. 389.1900; found 389.1898.

Preparation of *N,N'*-dibutyl 9-*exo*-10-*endo*-1-methylsulfanyl-8-phenyl-11-oxatricyclo[6.2.1.0^{2,7}]undeca-2,4,6-triene-9,10-dicarboxamide 4.45



n-Butyl lithium (0.67mL of a 1.6M solution in hexane, 1.068mmol.) was added dropwise to a stirred solution of diisopropylamine (0.15mL, 1.068mmol.) in THF at 0°C. The solution was stirred for ten minutes at 0°C and then cooled to -78°C. A solution of 3-phenyl thionophthalide (200mg, 0.89mmol.) in THF was added and the mixture was stirred for 1 hour at -78°C. Methyl iodide (0.066mL, 1.068mmol.) was added dropwise to the solution, which was then allowed to warm to room temperature over a period of 2 hours. *N,N'*-Dibutyl fumaramide (201mg, 0.89mmol.) in THF was added and the reaction was stirred overnight. The solvent was removed under reduced pressure to give a yellow oil. This was partitioned between ethyl acetate and water. The organic layer was dried (MgSO_4) and the

solvent removed under reduced pressure. This was purified by radial chromatography. Further impurities were removed by dissolving in dichloromethane and filtering to give *N,N'*-dibutyl 9-*exo*-10-*endo*-1-methylsulfanyl-8-phenyl-11-oxatricyclo[6.2.1.0^{2,7}]undeca-2,4,6-triene-9,10-dicarboxamide as a yellow solid (105mg, 25%).

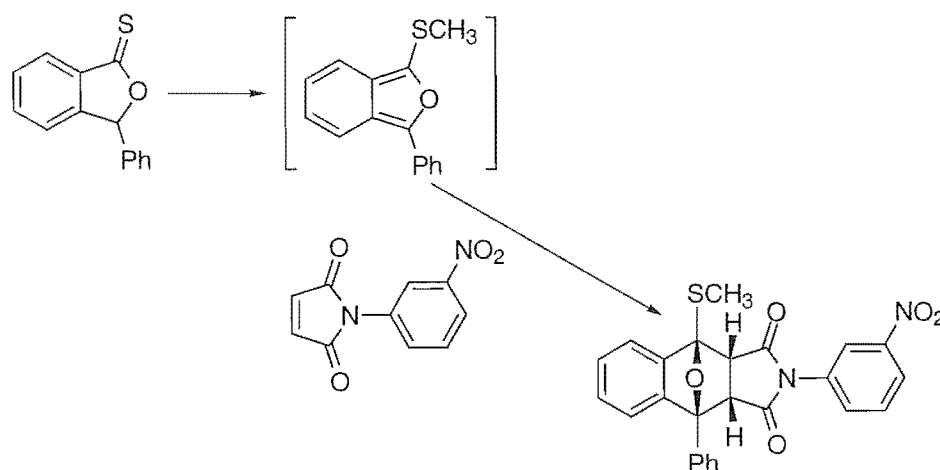
m.p. 202-205°C.

¹H NMR (CDCl₃) δ 0.77 (m, 3H, CH₃), 0.85-0.97 (m, 4H, CH₂CH₃), 1.04 (m, 3H, CH₃), 1.25-1.52 (m, 4H, NHCH₂CH₂), 2.33 (s, 3H, SCH₃), 2.90-3.12 (m, 4H, NHCH₂), 3.36 (d, 1H, J=4.9Hz, COCH), 3.66 (d, 1H, J=4.4Hz, COCH), 7.17-7.25 (m, 3H, aryl CH), 7.34-7.47 (m, 4H, aryl CH), 7.59 (d, 1H, J=8.3Hz, aryl CH), 7.657 (d, 1H, J=8.3Hz, aryl CH).

m/z (EI) 466.2 (M⁺), 393.1, 346.1, 277.1, 240.1.

High Res. C₂₇H₃₄N₂SO₃ calc. 466.22902; found 466.22896.

Preparation of 9-*endo*-13-*endo*-1-methylsulfanyl-8-phenyl-11-(3-nitrophenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7},7.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione 4.48



n-Butyl lithium (0.65mL of a 1.6M solution in hexane, 1.00mmol.) was added dropwise to a stirred solution of diisopropylamine (0.14mL, 1.00mmol.) in THF at

0°C. The solution was stirred for ten minutes at 0°C and then cooled to -78°C. A solution of 3-phenyl-1(3H)-isobenzofuranthione (180mg, 0.802mmol.) in THF was added and the mixture was stirred for 1 hour at -78°C. Methyl iodide (0.065mL, 1.00mmol.) was added dropwise to the solution, which was then allowed to warm to room temperature over a period of 2 hours. *N*-(3-Nitrophenyl)maleimide (175mg, 0.802mmol.) in THF was added and the reaction was stirred overnight. The solvent was removed under reduced pressure to give a brown oil. This was partitioned between ethyl acetate and water. The organic layer was dried (MgSO₄) and the solvent removed under reduced pressure. Purification by radial silica chromatography gave 9-*endo*-13-*endo*-1-methylsulfanyl-8-phenyl-11-(3-nitrophenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione as a clear oil (20.3mg, 6%).

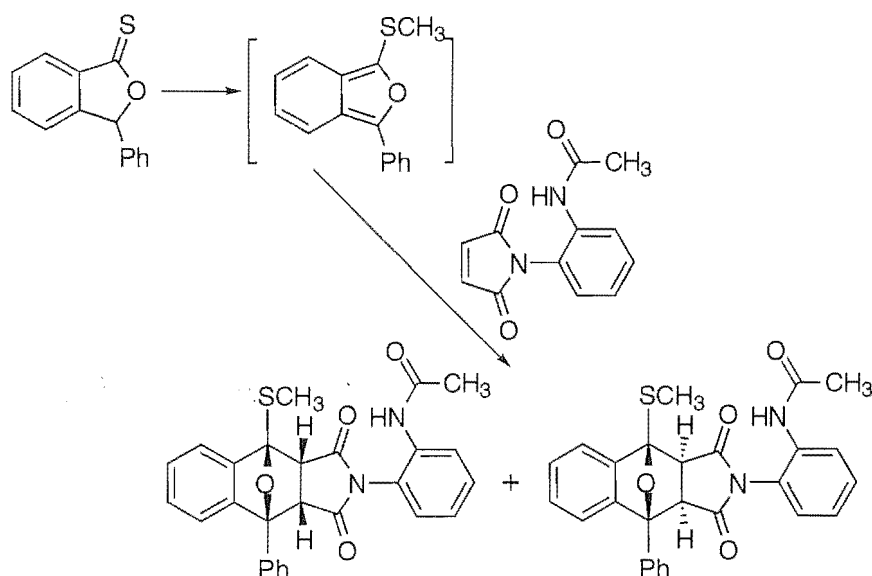
¹H NMR (CDCl₃) δ 2.37 (s, 3H, SCH₃), 4.00 (d, 1H, J=8.3Hz, COCH), 4.25 (d, 1H, J=8.8Hz, COCH), 6.99 (d, 1H, J=7.8Hz, aryl CH), 7.07 (d, 1H, J=7.4Hz, aryl CH), 7.12 (d, 1H, J=6.4Hz, aryl CH), 7.29-7.81 (m, 5H, aryl CH), 7.93 (m, 2H, aryl CH), 8.15 (d, 1H, J=7.4Hz, aryl CH), 8.23 (d, 1H, J=7.4Hz, aryl CH), 8.55 (d, 1H, J=8.3Hz, aryl CH).

m/z (EI) 458 (M⁺), 412 (M-NO₂⁺).

High Res. C₂₅H₁₈N₂SO₅ *m/z* calc. 458.0937; found 458.0962.

Preparation of 9-*endo*-13-*endo*-1-methylsulfanyl-8-phenyl-11-(2-acetamidophenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]

tetradeca-2,4,6-triene-10,12-dione 4.49 and 9-*exo*-13-*exo*-1-methylsulfanyl-8-phenyl-11-(2-acetamidophenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}] tetradeca-2,4,6-triene-10,12-dione 4.51



n-Butyl lithium (1.0mL of a 1.6M solution in hexane, 1.54mmol.) was added dropwise to a stirred solution of diisopropylamine (0.22mL, 1.54mmol.) in THF at 0°C. The solution was stirred for ten minutes at 0°C and then cooled to -78°C. A solution of 3-phenyl-1(3H)-isobenzofuranthione (290mg, 1.29mmol.) in THF was added and the mixture was stirred for 1 hour at -78°C. Methyl iodide (0.10mL, 1.54mmol.) was added dropwise to the solution, which was then allowed to warm to room temperature over a period of 2 hours. *N*-(2-Acetylaminophenyl)maleimide (296mg, 1.29mmol.) in dichloromethane was added and the reaction was stirred overnight. The solvent was removed under reduced pressure to give an oil which was partitioned between ethyl acetate and water. The organic layer was then dried (MgSO₄) and the solvent removed under reduced pressure. This was purified by radial silica chromatography to give 9-*endo*-13-*endo*-1-methylsulfanyl-8-phenyl-11-

(2-acetamidophenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione **4.49** as a white solid (80.0mg, 13.2%)

m.p. 213-215°C.

¹H NMR (CDCl₃) δ 1.95 (s, 3H, CH₃), 2.33 (s, 3H, CH₃), 3.98 (d, 1H, J=8.3Hz, COCH), 4.20 (d, 1H, J=8.3Hz, COCH), 5.65 (d, 1H, J=7.8Hz, aryl CH), 6.92 (t, 1H, J=7.8Hz, aryl CH), 7.04 (d, 1H, J=7.3Hz, aryl CH), 7.21-7.52 (m, 7H, aryl CH), 7.62 (d, 1H, J=8.3Hz, aryl CH), 7.90 (m, 2H, aryl CH).

m/z (EI) 470 (M⁺), 428 (M-COCH₂⁺).

High Res. C₂₇H₂₂N₂SO₄ *m/z* calc. 470.13004; found 470.12996.

IR *v*_{max} 3018.4, 1716.5, 1510.2, 1450.4, 1377.1, 1236.3, 1199.6 cm⁻¹.

and 9-*exo*-13-*exo*-1-methylsulfanyl-8-phenyl-11-(2-acetamidophenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione **4.51** as a pale yellow solid (7.1mg, 1.2%).

m.p. 215°C (decomp.).

¹H NMR (CDCl₃) δ 2.23 (s, 3H, CH₃), 2.27 (s, 3H, CH₃), 3.38 (d, 1H, J=6.4Hz, COCH), 3.68 (d, 1H, J=6.4Hz, COCH), 7.07-7.52 (m, 11H, aryl CH), 7.59 (d, 1H, J=6.8Hz, aryl CH), 8.31 (d, 1H, J=8.3Hz, aryl CH).

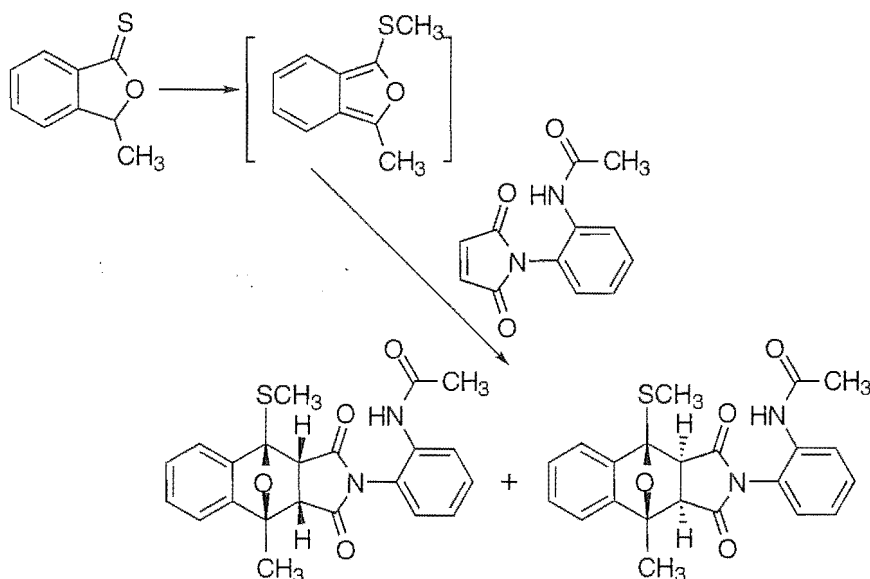
m/z (EI) 470 (M⁺), 428 (M-COCH₂⁺).

High Res. C₂₇H₂₂N₂SO₄ *m/z* calc. 470.13004; found 470.12989.

¹³C NMR (CDCl₃) δ 12.22, 53.67, 53.90, 54.22, 88.96, 100.08, 107.27, 110.09, 119.96, 121.96, 123.73, 125.28, 127.57, 127.97, 128.11, 129.82, 132.34.

IR *v*_{max} 3018.4, 1720.4, 1525.6, 1458.1, 1382.9, 1301.9, 1236.3, 1195.8 cm⁻¹.

Preparation of 9-*endo*-13-*endo*-1-methylsulfanyl-8-methyl-11-(2-acetamidophenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione 4.50 and 9-*exo*-13-*exo*-1-methylsulfanyl-8-methyl-11-(2-acetamidophenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione 4.52



n-Butyl lithium (0.26mL of a 1.6M solution in hexane, 0.413mmol.) was added dropwise to a stirred solution of diisopropylamine (0.06mL, 0.413mmol.) in THF at 0°C. The solution was stirred for ten minutes at 0°C and then cooled to -78°C. A solution of 3-methyl-1(3H)-isobenzofuranthione (61.7mg, 0.376mmol.) in THF was added and the mixture was stirred for 1 hour at -78°C. Methyl iodide (0.0024mL, 0.413mmol.) was added dropwise to the solution, which was then allowed to warm to room temperature over a period of 2 hours. *N*-(2-Acetylaminophenyl)maleimide (86.4mg, 0.376mmol.) in dichloromethane was added and the reaction was stirred overnight. The solvent was removed under reduced pressure to give an oil which was partitioned between ethyl acetate and water. The organic layer was dried (MgSO₄) and the solvent removed under

reduced pressure. This was purified by radial silica chromatography to give 9-*endo*-13-*endo*-1-methylsulfanyl-8-methyl-11-(2-acetamidophenyl)-11-aza-14-oxatetracyclo [6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione **4.50** as fine white crystals (40.9mg, 26.6%)

m.p. 279-280°C.

Analysis C₂₂H₂₀N₂SO₄ calc. C, 64.69; H, 4.94; N, 6.86; found C, 64.95; H, 4.73; N, 6.80.

¹H NMR (CDCl₃) δ 2.10 (s, 3H, CH₃), 2.16 (s, 3H, NHCOCH₃), 2.17 (s, 3H, SCH₃), 3.12 (d, 1H, J=6.4Hz, COCH), 3.24 (d, 1H, J=6.4Hz, COCH), 7.18-7.22 (m, 2H, aryl CH), 7.36-7.49 (m, 5H, aryl CH), 8.40 (d, 1H, J=8.3Hz, aryl CH).

m/z (EI) 408 (M⁺), 366 (M-COCH₂⁺).

High Res. C₂₂H₂₀N₂SO₄ *m/z* calc. 408.11445; found 408.11495.

¹³C NMR (CDCl₃) δ 12.05, 14.12, 24.10, 53.87, 54.21, 94.57, 110.09, 118.71, 119.92, 122.01, 123.86, 127.81, 127.90, 128.18, 130.02, 133.90, 162.99.

IR *v*_{max} 3022.2, 1718.5, 1529.4, 1458.1, 1384.8, 1190.0 cm⁻¹.

and 9-*exo*-13-*exo*-1-methylsulfanyl-8-methyl-11-(2-acetamidophenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione **4.52** as a white solid (1.7mg, 1.1%).

m.p. 280-281°C.

¹H NMR (CDCl₃) δ 1.96 (s, 3H, CH₃), 2.07 (s, 3H, NHCOCH₃), 2.26 (s, 3H, SCH₃), 3.72 (d, 1H, J=7.8Hz, COCH), 3.86 (d, 1H, J=7.8Hz, COCH), 5.65 (d, 1H, J=7.9Hz, aryl CH), 6.95 (t, 1H, J=7.8Hz, aryl CH), 7.28 (m, 3H, aryl CH), 7.39 (m, 2H, aryl CH), 7.63 (d, 1H, J=7.8Hz, aryl CH).

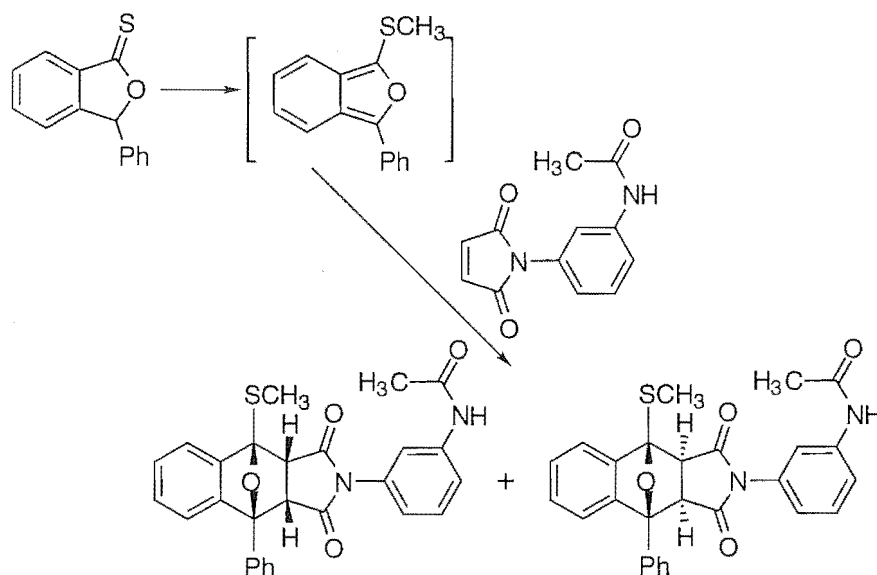
m/z (EI) 408 (M⁺), 366 (M-COCH₂⁺).

High Res. C₂₂H₂₀N₂SO₄ *m/z* calc. 408.11445; found 408.11501.

^{13}C NMR (CDCl_3) δ 11.83, 16.94, 53.86, 54.77, 61.94, 77.54, 87.08, 101.17, 113.70, 119.85, 120.93, 124.87, 125.18, 126.69, 128.00, 128.32, 129.09, 153.47.

IR ν_{max} 3024.2, 1716.5, 1514.0, 1456.2, 1375.2, 1236.3, 1180.4 cm^{-1} .

Preparation of 9-endo-13-endo-1-methylsulfanyl-8-phenyl-11-(3-acetamidophenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}] tetradeca-2,4,6-triene-10,12-dione 4.53 and 9-exo-13-exo-1-methylsulfanyl-8-phenyl-11-(3-acetamidophenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}] tetradeca-2,4,6-triene-10,12-dione 4.55



n-Butyl lithium (1.0mL of a 1.6M solution in hexane, 1.54mmol.) was added dropwise to a stirred solution of diisopropylamine (0.22mL, 1.54mmol.) in THF at 0°C. The solution was stirred for ten minutes at 0°C and then cooled to -78°C using a dry-ice and acetone bath. A solution of 3-phenyl-1(3H)-isobenzofuranthione (290mg, 1.29mmol.) in THF was added and the mixture was stirred for 1 hour at -78°C. Methyl iodide (0.10mL, 1.54mmol.) was added dropwise to the solution,

which was then allowed to warm to room temperature over a period of 2 hours. *N*-(3-Acetylaminophenyl)maleimide (296mg, 1.29mmol.) in dichloromethane was added and the reaction was stirred overnight. The solvent was removed under reduced pressure to give an oil which was partitioned between ethyl acetate and water. The organic layer was dried (MgSO₄) and the solvent removed under reduced pressure. This was purified by radial silica chromatography to give both 9-*endo*-13-*endo*-1-methylsulfanyl-8-phenyl-11-(3-acetamidophenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione **4.53** as a yellow oil (78.4mg, 12.9%)

¹H NMR (CDCl₃) δ 2.02 (s, 1H, CH₃), 2.11 (s, 1H, CH₃), 2.35 (s, 1H, CH₃), 3.96 (d, 1H, J=8.3Hz, COCH), 4.20 (d, 1H, J=8.3Hz, COCH), 5.98 (d, 1H, J=7.4Hz, aryl CH), 6.84 (s, 1H, aryl CH), 7.00-7.09 (m, 3H, aryl CH), 7.27-7.52 (m, 4H, aryl CH), 7.61 (m, 1H aryl CH), 7.68 (m, 1H, aryl CH), 7.93 (d, 2H, aryl CH).

m/z (EI) 470 (M⁺), 428 (M-COCH₂⁺).

High Res. C₂₇H₂₂N₂SO₄ *m/z* calc. 470.13004; found 470.12994.

¹³C NMR (CDCl₃) δ 12.23, 52.27, 53.64, 54.07, 94.09, 110.70, 115.25, 115.55, 116.89, 119.09, 120.70, 120.85, 121.10, 124.69, 126.45, 128.05, 128.11, 128.25, 128.50, 128.66, 129.04, 135.20, 139.35.

IR ν_{\max} 3018.4, 1716.5, 1693.4, 1608.5, 1529.4, 1490.9, 1236.3, 1222.8 cm⁻¹.

and 9-*exo*-13-*exo*-1-methylsulfanyl-8-phenyl-11-(3-acetamidophenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione **4.55** as a yellow oil (2.0mg, 0.3%).

¹H NMR (CDCl₃) δ 2.05 (s, 1H, CH₃), 2.15 (s, 1H, CH₃), 2.36 (s, 1H, CH₃), 4.34 (d, 1H, J=5.8Hz, COCH), 4.75 (d, 1H, J=5.8Hz, COCH), 6.85 (s,

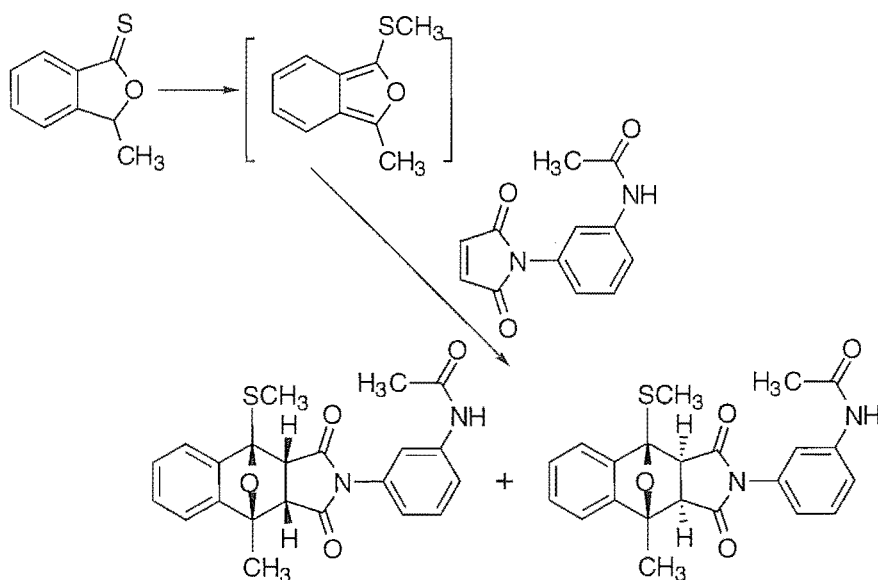
1H, aryl CH), 6.96 (d, 1H, $J=9.3\text{Hz}$, aryl CH), 7.10-7.59 (m, 9H, aryl CH), 7.97 (d, 1H, $J=8.3\text{Hz}$, aryl CH), 8.20 (d, 1H, $J=9.3\text{Hz}$, aryl CH).

m/z (EI) 470 (M^+), 428 ($M-\text{COCH}_2^+$).

High Res. $\text{C}_{27}\text{H}_{22}\text{N}_2\text{SO}_4$ m/z calc. 470.13004; found 470.13026.

IR ν_{max} 3018.4, 1720.4, 1608.5, 1529.4, 1492.8, 1379.0, 1344.3, 1236.3 cm^{-1} .

Preparation of 9-endo-13-endo-1-methylsulfanyl-8-methyl-11-(3-acetamidophenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione 4.54 and 9-exo-13-exo-1-methylsulfanyl-8-methyl-11-(3-acetamidophenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione 4.56



n-Butyl lithium (0.26mL of a 1.6M solution in hexane, 0.413mmol.) was added dropwise to a stirred solution of diisopropylamine (0.06mL, 0.413mmol.) in THF at 0°C. The solution was stirred for ten minutes at 0°C and then cooled to -78°C. A solution of 3-methyl-1(3H)-isobenzofuranthione (61.7mg, 0.376mmol.) in THF

was added and the mixture was stirred for 1 hour at -78°C . Methyl iodide (0.0024mL, 0.413mmol.) was added dropwise to the solution, which was then allowed to warm to room temperature over a period of 2 hours. *N*-(3-Acetylaminophenyl)maleimide (86.4mg, 0.376mmol.) in dichloromethane was added and the reaction was stirred overnight. The solvent was removed under reduced pressure to give an oil which was partitioned between ethyl acetate and water. The organic layer was dried (MgSO_4) and the solvent removed under reduced pressure. This was purified by radial silica chromatography to give both 9-*endo*-13-*endo*-1-methylsulfanyl-8-methyl-11-(3-acetamidophenyl)-11-aza-14-oxatetracyclo [6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione **4.54** as a pale yellow oil (77.7mg, 50.6%).

^1H NMR (CDCl_3) δ 2.01 (s, 1H, CH_3), 2.08 (s, 1H, NHCOCH_3), 2.27 (s, 1H, SCH_3), 3.67 (d, 1H, $J=8.8\text{Hz}$, COCH), 3.81 (d, 1H, $J=8.8\text{Hz}$, COCH), 5.92 (m, 1H aryl CH), 6.92 (s, 1H, aryl CH), 7.04 (m, 2H, aryl CH), 7.28-7.44 (m, 4H, aryl CH).

m/z (EI) 408 (M^+), 366 ($\text{M}-\text{COCH}_2^+$).

High Res. $\text{C}_{22}\text{H}_{20}\text{N}_2\text{SO}_4$ m/z calc. 408.11445; found 408.11468.

^{13}C NMR (CDCl_3) δ 11.86, 16.95, 23.96, 53.87, 54.65, 86.39, 94.31, 116.85, 118.98, 119.77, 120.81, 120.91, 128.01, 128.40, 128.52, 130.64, 138.16, 139.91, 143.18, 167.58, 171.40, 172.29.

IR ν_{max} 1716.5, 1529.4, 1492.8, 1444.6, 1377.1, 1236.3, 1197.7, 1186.1 cm^{-1} .

and 9-*exo*-13-*exo*-1-methylsulfanyl-8-methyl-11-(3-acetamidophenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione **4.56** as a pale yellow oil (21.5mg, 14.0%).

^1H NMR (CDCl_3) δ 2.01 (s, 3H, CH_3), 2.08 (s, 3H, NHCOCH_3), 2.13 (s, 3H, SCH_3), 3.05 (d, 1H, $J=6.4\text{Hz}$, COCH), 3.19 (d, 1H, $J=6.4\text{Hz}$, COCH),

7.02 (d, 1H, $J=7.8\text{Hz}$, aryl CH), 7.27-7.43 (m, 5H, aryl CH), 7.53 (s, 1H, aryl CH), 7.94 (s, 1H, aryl CH).

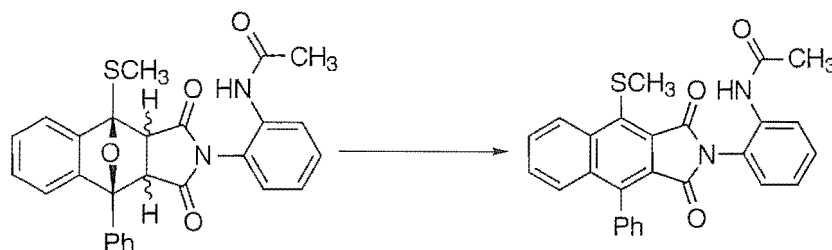
m/z (EI) 408 (M^+), 366 ($M-\text{COCH}_2^+$).

High Res. $\text{C}_{22}\text{H}_{20}\text{N}_2\text{SO}_4$ m/z calc. 408.11445; found 408.11499.

^{13}C NMR (CDCl_3) δ 172.67, 171.67, 146.74, 142.84, 139.55, 128.98, 127.85, 127.57, 121.43, 119.91, 119.38, 118.47, 117.01, 111.63, 94.12, 85.83, 71.24, 54.07, 53.60, 24.00, 14.10, 11.99.

IR ν_{max} 1714.6, 1606.6, 1492.8, 1444.6, 1380.9, 1197.7 cm^{-1} .

Preparation of *N*-(2-acetamidophenyl) 1-methylsulfanyl-8-phenyl-naphthalene-2,3-dicarboximide 4.57



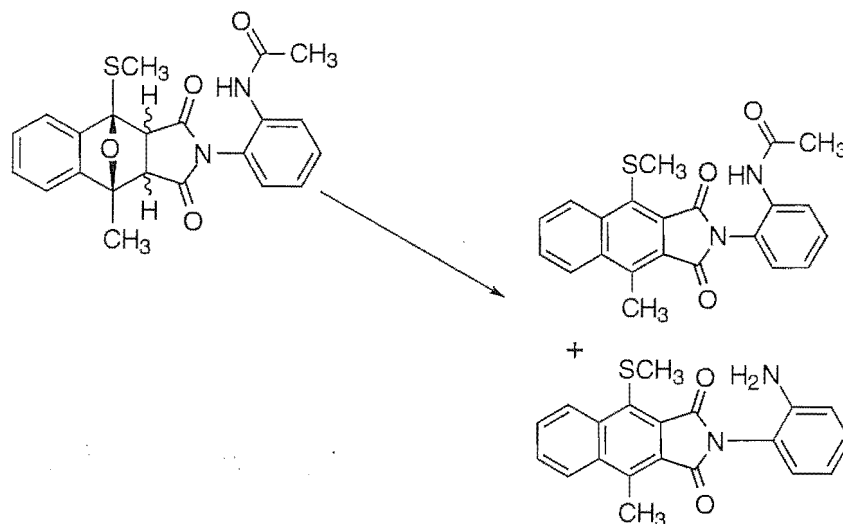
1-Methylsulfanyl-8-phenyl-11-(2-acetamidophenyl)-11-aza-14-oxatetracyclo [6.5.1.0^{2,7}.1³]tetradeca-2,4,6-triene-10,12-dione (6.0mg, 0.0132mmol.) was dissolved in acetonitrile and concentrated hydrochloric acid added dropwise to a pH of 1. This solution was then stirred for 2 days after which the solvent was removed under reduced pressure to give an oil. This was purified by radial silica chromatography to give *N*-(2-acetamidophenyl) 1-methylsulfanyl-8-phenyl-naphthalene-2,3-dicarboximide as a pale yellow oil (2.0mg, 34%).

^1H NMR (CDCl_3) δ 2.05 (s, 3H, CH_3), 2.69 (s, 3H, SCH_3), 7.28-7.67 (m, 9H, aryl CH), 7.79 (m, 3H, aryl CH), 9.07 (d, 1H, $J=8.3\text{Hz}$, aryl CH).

m/z (EI) 452.1 (M^+), 410.1 ($M-\text{COCH}_2^+$), 377.1.

High Res. $\text{C}_{27}\text{H}_{20}\text{N}_2\text{SO}_3$ m/z calc. 452.11947; found 452.11927.

Preparation of *N*-(2-acetamidophenyl) 1-methylsulfanyl-8-methyl-naphthalene-2,3-dicarboximide 4.58 and *N*-(2-aminophenyl) 1-methylsulfanyl-8-methyl-naphthalene-2,3-dicarboximide 4.59



1-Methylsulfanyl-8-methyl-11-(2-acetamidophenyl)-11-aza-14-oxatetracyclo [6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione (9.5mg, 0.0233mmol.) was dissolved in acetonitrile and concentrated hydrochloric acid added dropwise until a pH of 1 was reached. This solution was then stirred for 5 days and monitored by TLC, after which the solvent was removed under reduced pressure to give an oil. This was purified by radial silica chromatography to give *N*-(2-acetamidophenyl) 1-methylsulfanyl-8-methyl-naphthalene-2,3-dicarboximide **4.58** as a pale brown oil (4.0mg, 44%)

¹H NMR (CDCl₃) δ 2.08 (s, 3H, COCH₃), 2.62 (s, 3H, SCH₃), 3.16 (s, 3H, CH₃), 6.93 (t, 1H, J=8.3Hz, aryl CH), 7.19 (d, 1H, J=8.3Hz, aryl CH), 7.30-7.70 (m, 6H, aryl CH).

m/z (EI) 390.1 (M⁺), 348.1 (M-COCH₂⁺).

High Res. C₂₂H₁₈N₂SO₃ *m/z* calc. 390.10382 ; found 390.10382.

IR *v*_{max} (CDCl₃) 2927.7, 1762.8, 1708.8, 1515.9, 1456.2, 1379.0, 1236.3, 1197.7, 1136.0 cm⁻¹.

and *N*-(2-aminophenyl) 1-methylsulfanyl-8-methyl-naphthalene-2,3-dicarboximide **4.59** as a yellow solid (1.1mg 14%).

m.p. 225-227°C.

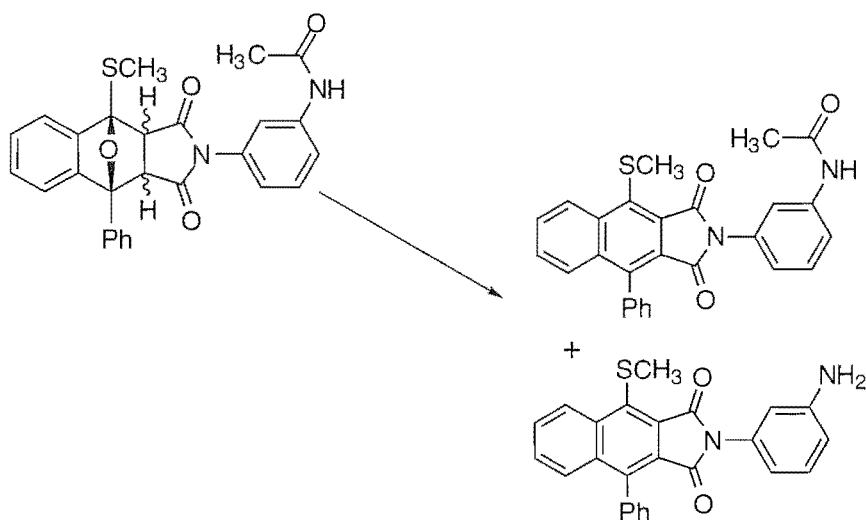
^1H NMR (CDCl_3) δ 2.62 (s, 3H, SCH₃), 3.16 (s, 3H, CH₃), 6.93 (m, 2H, aryl CH), 7.19 (d, 1H, $J=6.9\text{Hz}$, aryl CH), 7.28 (m, 1H, aryl CH), 7.81 (m, 2H, aryl CH), 8.32 (d, 1H, $J=7.3\text{Hz}$, aryl CH), 9.06 (d, 1H, $J=7.3\text{Hz}$, aryl CH).

m/z (EI) 348.1 (M^+), 297.1.

High Res. $\text{C}_{20}\text{H}_{16}\text{N}_2\text{SO}_2$ m/z calc. 348.09325 ; found 348.09349.

IR ν_{max} (CDCl_3) 1760.9, 1708.8, 1504.4, 1380.9, 1234.4, 1218.9, 1199.6 cm^{-1} .

Preparation of *N*-(3-acetamidophenyl) 1-methylsulfanyl-8-phenyl-naphthalene-2,3-dicarboximide **4.60 and *N*-(3-aminophenyl) 1-methylsulfanyl-8-phenyl-naphthalene-2,3-dicarboximide **4.62****



1-Methylsulfanyl-8-phenyl-11-(3-acetamidophenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione (15.0mg, 0.0319mmol.) was dissolved in acetonitrile and concentrated hydrochloric acid added dropwise until a pH of 1 was reached. This solution was then stirred for 5 days and monitored by

TLC, after which the solvent was removed under reduced pressure to give an oil. This was purified by radial silica chromatography to give both *N*-(3-acetamidophenyl) 1-methylsulfanyl-8-phenyl-naphthalene-2,3-dicarboximide **4.60** as a yellow oil (6mg, 41%)

^1H NMR (CDCl_3) δ 2.15 (s, 3H, CH_3), 2.70 (s, 3H, SCH_3), 7.01 (m, 1H, aryl CH), 7.22 (m, 1H, aryl CH), 7.30 (d, 1H, $J=8.3\text{Hz}$, aryl CH), 7.37-7.44 (m, 2H, aryl CH), 7.52-7.70 (m, 4H, aryl CH), 7.81 (t, 2H, $J=7.7\text{Hz}$, aryl CH), 7.99 (d, 1H, $J=7.9\text{Hz}$, aryl CH), 9.10 (d, 1H, $J=8.3\text{Hz}$, aryl CH).

m/z (EI) 452.1 (M^+), 410.1 ($\text{M}-\text{COCH}_2^+$), 377.1.

High Res. $\text{C}_{27}\text{H}_{20}\text{N}_2\text{SO}_3$ m/z calc. 452.11947; found 452.11902.

and *N*-(3-aminophenyl) 1-methylsulfanyl-8-phenyl-naphthalene-2,3-dicarboximide **4.62** as a yellow oil (2mg, 15%).

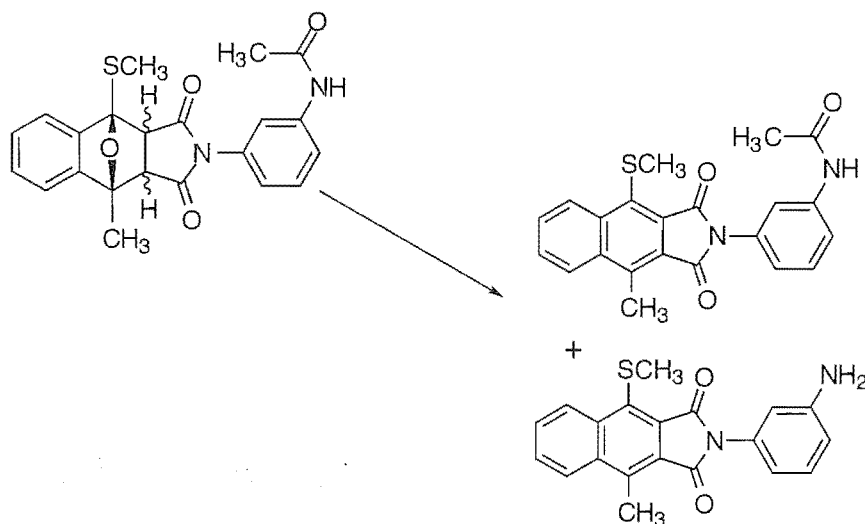
^1H NMR (CDCl_3) δ 2.70 (s, 3H, SCH_3), 6.67 (d, 1H, $J=7.8\text{Hz}$, aryl CH), 6.76 (s, 1H, aryl CH), 6.84 (d, 1H, $J=7.9\text{Hz}$, aryl CH), 7.22 (t, 1H, $J=8.3\text{Hz}$, aryl CH), 7.39 (m, 2H, aryl CH), 7.54 (m, 3H, aryl CH), 7.64 (t, 1H, $J=8.3\text{Hz}$, aryl CH), 7.80 (t, 2H, $J=7.8\text{Hz}$, aryl CH), 9.09 (d, 1H, $J=8.3\text{Hz}$, aryl CH).

m/z (EI) 410.1 (M^+), 377.1.

High Res. $\text{C}_{25}\text{H}_{18}\text{N}_2\text{SO}_2$ m/z calc. 410.10890 ; found 410.10967.

IR ν_{max} (CDCl_3) 3030.0, 3018.4, 1716.5, 1379.0, 1222.8, 1215.1 cm^{-1} .

Preparation of *N*-(3-acetamidophenyl) 1-methylsulfanyl-8-methyl-naphthalene-2,3-dicarboximide **4.61 and *N*-(3-aminophenyl) 1-methylsulfanyl-8-methyl-naphthalene-2,3-dicarboximide **4.63****



1-Methylsulfanyl-8-methyl-11-(3-acetamidophenyl)-11-aza-14-oxatetracyclo [6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione (16.4mg, 0.0402mmol.) was dissolved in acetonitrile and concentrated hydrochloric acid added dropwise until a pH of 1 was reached. This solution was then stirred for 5 days and monitored by TLC, after which the solvent was removed under reduced pressure to give an oil. This was purified by radial silica chromatography to give *N*-(3-acetamidophenyl) 1-methylsulfanyl-8-methyl-naphthalene-2,3-dicarboximide **4.61** as a pale yellow solid (5.3mg, 34%)

m.p. 155-156°C.

¹H NMR (CDCl₃) δ 2.18 (s, 3H, COCH₃), 2.61 (s, 3H, SCH₃), 3.16 (s, 3H, CH₃), 7.38 (m, 1H, aryl CH), 7.48 (t, 1H, J=7.8Hz, aryl CH), 7.56 (d, 1H, J=7.8Hz, aryl CH), 7.70 (s, 1H, aryl CH), 7.80 (m, 2H, aryl CH), 8.32 (d, 1H, J=7.8Hz, aryl CH), 9.06 (d, 1H, J=7.8Hz, aryl CH).

m/z (EI) 390.1 (M⁺), 348.1 (M-COCH₂⁺), 315.1.

High Res. C₂₂H₁₈N₂SO₃ *m/z* calc. 390.10382 ; found 390.10346.

IR ν_{\max} (CDCl₃) 3018.4, 1762.8, 1708.8, 1606.6, 1492.8, 1419.5, 1382.9, 1234.4, 1207.4 cm⁻¹.

and *N*-(3-aminophenyl) 1-methylsulfanyl-8-methyl-naphthalene-2,3-dicarboximide **4.63** as a red-brown solid (0.8mg, 6%).

m.p. 166-168°C.

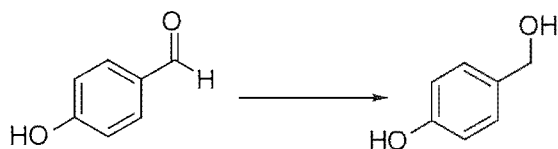
¹H NMR (CDCl₃) δ 2.62 (s, 3H, SCH₃), 3.17 (s, 3H, CH₃), 6.74 (d, 1H, J=8.3Hz, aryl CH), 6.81 (s, 1H, aryl CH), 6.87 (d, 1H, J=7.3Hz, aryl CH), 7.30 (t, 1H, J=7.8Hz, aryl CH), 7.80 (m, 2H, aryl CH), 8.31 (d, 1H, J=6.9Hz, aryl CH), 9.06 (d, 1H, J=6.9Hz, aryl CH).

m/z (EI) 348.1 (M⁺), 315.1.

High Res. C₂₀H₁₆N₂SO₂ *m/z* calc. 348.09325; found 348.09364.

IR ν_{\max} (CDCl₃) 3018.4, 1708.8, 1234.4, 1199.6 cm⁻¹.

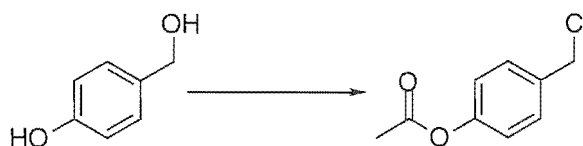
Preparation of 4-hydroxybenzyl alcohol **4.68**



4-Hydroxybenzaldehyde (10g, 81.9mmol.) was dissolved in methanol (10mL) and added dropwise to a solution of NaBH₄ in methanol (10%, 10mL). The pH was adjusted to 7 and the solution was stirred until the reaction was complete. The solvent was removed under reduced pressure giving a pink solid. This was washed with a little cold water and recrystallised from water to give 4-hydroxybenzyl alcohol as pale pink crystals (10.17g, 100%).

m.p. 120-122°C.

¹H NMR (CD₃OD) δ 4.47 (s, 2H, CH₂), 6.73-6.75 (m, 2H, aryl CH), 7.15-7.17 (m, 2H, aryl CH).

Preparation of 4-chloromethylphenyl acetate 4.69

Acetyl chloride (50mL) was stirred in an open flask, cooled in an ice-bath. 4-Hydroxybenzyl alcohol (11.45g, 92.2mmol.) was slowly added, causing HCl gas to be evolved. The mixture was left to stir overnight to allow the excess acetyl chloride to evaporate. The resulting solution was neutralised with NaHCO₃. This was then transferred to a separating funnel and extracted twice with ethyl acetate. The combined organic extracts were dried over MgSO₄, filtered, and the solvent removed under reduced pressure to give a brown oil. This was distilled under vacuum (150°C, 1.5mmHg) to give 4-chloromethylphenyl acetate as a clear oil which solidified on cooling as a white powder (6.92g, 40%).

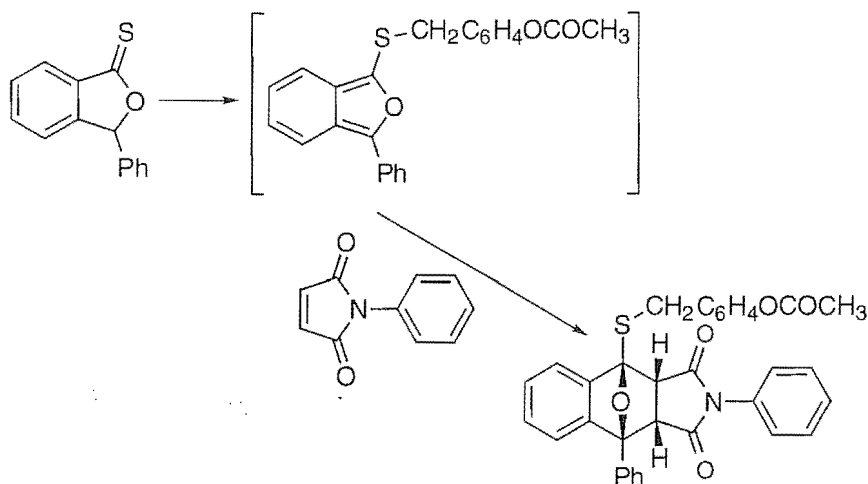
¹H NMR (CDCl₃) δ 2.28 (s, 3H, CH₃), 4.56 (s, 2H, CH₂), 7.07-7.10 (d, 2H, J=8.7Hz, aryl CH), 7.39-7.42 (d, 2H, J=8.3Hz, aryl CH).

m/z (EI) 184 (M⁺), 142, 107.

IR ν_{max} (KBr) 3024, 1762 cm⁻¹.

Preparation of 9-endo-13-endo-1-(4-acetoxyphenyl)methylsulfanyl-8,11-diphenyl-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione

4.71



n-Butyl lithium (0.38mL of a 1.6M solution in hexane, 0.61mmol.) was added dropwise to a stirred solution of diisopropylamine (0.086mL, 0.61mmol.) in THF at 0°C. The solution was stirred for ten minutes at 0°C and then cooled to -78°C. A solution of 3-phenyl-1(3H)-isobenzofuranthione (115mg, 0.51mmol.) in THF was added and the mixture was stirred for 2 hours at -78°C, during which time it turned red. 4-Chloromethylphenyl acetate (94mg 0.51mmol.) in THF was added dropwise to the solution, which was then allowed to warm to room temperature over a period of 6 hours. *N*-Phenyl maleimide (88mg, 0.51mmol.) in THF was added and the reaction was stirred overnight. The solvent was removed under reduced pressure to give an oil which was partitioned between ethyl acetate and water. The organic layer was dried (MgSO₄) and the solvent removed under reduced pressure. This was purified by radial chromatography to give 9-endo-13-endo-1-(4-acetoxyphenyl)methylsulfanyl-8,11-diphenyl-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione as white needles (6mg, 2%).

m.p. 210-211°C.

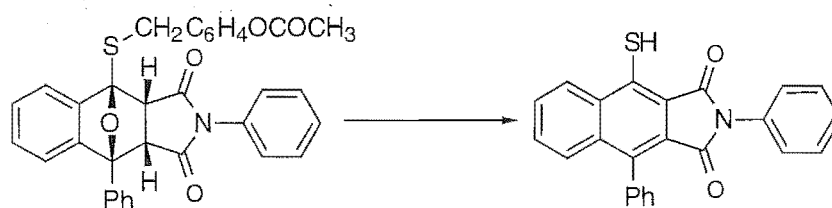
^1H NMR (CD_3Cl_3) δ 2.30 (s, 3H, CH_3), 3.96 (d, 1H, $J=8.8\text{Hz}$, COCH), 4.08 (d, $J=13.2\text{Hz}$, CH_2), 4.16 (d, 1H, $J=8.8\text{Hz}$, COCH), 6.46-6.49 (m, 2H, aryl CH), 7.00-7.05 (m, 3H, aryl CH), 7.26-7.29 (m, 3H, aryl CH), 7.31-7.39 (m, 4H, aryl CH), 7.41-7.53 (m, 4H, aryl CH), 7.88 (d, 2H, $J=8.3\text{Hz}$, aryl CH).

m/z (EI) 548 (M^+), 374.

High Res $\text{C}_{33}\text{H}_{25}\text{NSO}_5$ m/z calc. 547.1454; found 547.1464.

IR ν_{max} (KBr) 1706, 1470, 1320 cm^{-1} .

Preparation of *N*-phenyl 1-mercapto-4-phenyl-naphthalene-2,3-dicarboximide 4.72



9-*endo*-13-*endo*-1-(4-Acetoxyphenyl)methylsulfanyl-8,11-diphenyl-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione (3mg, 0.0055mmol.) was added to methanol (1mL) containing sodium bicarbonate (0.8mg, 0.0094mmol.) and refluxed for an hour and monitored by TLC, during which time the solution became yellow. The solvent was removed under reduced pressure to give a yellow solid. This was partitioned between ethyl acetate and water. The ethyl acetate fractions were combined, dried (MgSO_4), and the ethyl acetate removed under reduced pressure. This was purified by radial silica chromatography and recrystallised from dichloromethane to give *N*-phenyl 1-mercapto-4-phenyl-naphthalene-2,3-dicarboximide as yellow crystals (0.9mg, 42%).

m.p. 150-152°C.

^1H NMR (CDCl_3) δ 7.37-7.41 (m, 3H, aryl CH), 7.43-7.47 (m, 3H, aryl CH), 7.52-7.54 (m, 3H, aryl CH), 7.62-7.67 (m, 1H, aryl CH), 7.75-7.82 (m, 3H, aryl CH), 8.35 (d, 1H, $J=7.8\text{Hz}$, aryl CH).

m/z (EI) 381 (M^+).

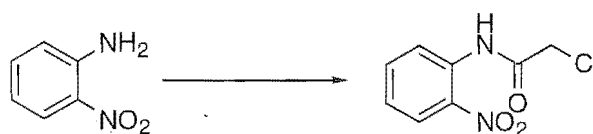
High Res $\text{C}_{24}\text{H}_{15}\text{NSO}_2$ m/z calc. 381.0824; found 381.0816.

IR ν_{max} (CDCl_3) 3018.4, 1768.6, 1718.5, 1500.5, 1379.0, 1234.4, 1199.6 cm^{-1} .

Part B Experimental

7.5 Chapter 5 Experimental

Preparation of *N*-(2-nitrophenyl) chloroacetamide 5.2



2-Nitroaniline (6g, 43.4mmol.) was dissolved in ethyl acetate (90mL). Chloroacetyl chloride (4.9g, 3.46mL, 43.3mmol.) was added by syringe and the resulting solution refluxed on an oil bath for 4 hours. The ethyl acetate was removed under reduced pressure to give *N*-(2-nitrophenyl) chloroacetamide as yellow needles (9.6g, 100%).

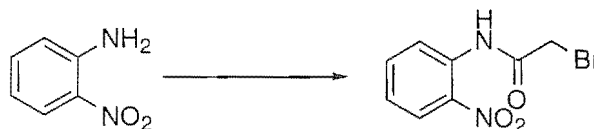
m.p. 78-80°C.

Analysis C₈H₇N₂O₃Cl calc. C, 44.77; H, 3.29; N, 13.06; found C, 44.76, H, 3.29, N, 13.09.

¹H NMR (CDCl₃) δ 4.26 (s, 2H, CH₂), 7.26 (t, 1H, J=7.8Hz, aryl CH) 7.67 (t, 1H, J=7.8Hz, aryl CH), 8.22 (d, 1H, J=7.8Hz, aryl CH), 8.73 (d, 1H, J=7.8Hz, aryl CH), 11.30 (br.s, 1H, NH).

m/z (EI) 214.0 (M⁺), 165.0, 138.0.

High Res. C₈H₇N₂O₃Cl calc. 214.01452; found 214.01446.

Preparation of *N*-(2-nitrophenyl) bromoacetamide 5.3

2-Nitroaniline (6g, 43.4mmol.) was dissolved in ethyl acetate (90mL). Bromoacetyl bromide (8.76g, 3.78mL, 43.4mmol.) was added by syringe and the resulting solution refluxed on an oil bath for 4 hours. The ethyl acetate was removed under reduced pressure to give *N*-(2-nitrophenyl) bromoacetamide as yellow crystals (11.24g, 100%).

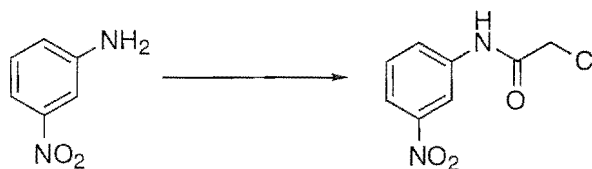
m.p. 60-61°C.

^1H NMR (CDCl_3) δ 4.08 (s, 2H, CH_2), 7.27, (t, 1H, $J=7.4\text{Hz}$, aryl CH), 7.70 (t, 1H, $J=7.4\text{Hz}$, aryl CH), 8.26 (d, 1H, $J=7.4\text{Hz}$, aryl CH), 8.74 (d, 1H, $J=7.4\text{Hz}$, aryl CH), 11.28 (br.s, 1H, NH).

m/z (EI) 260.0 (M^+), 138.0, 92.0.

High Res. $\text{C}_8\text{H}_7\text{N}_2\text{O}_3^{81}\text{Br}$ calc. 259.96209; found 259.96444.

High Res. $\text{C}_8\text{H}_7\text{N}_2\text{O}_3^{79}\text{Br}$ calc. 257.96400; found 257.96413.

Preparation of *N*-(3-nitrophenyl) chloroacetamide 5.5

3-Nitroaniline (6g, 43.4mmol.) was dissolved in ethyl acetate (90mL). Chloroacetyl chloride (4.9g, 3.46mL, 43.4mmol.) was added by syringe and the resulting solution refluxed on an oil bath for 4 hours. The ethyl acetate was removed under reduced pressure to give *N*-(3-nitrophenyl) chloroacetamide as pale brown crystals (9.6g, 100%).

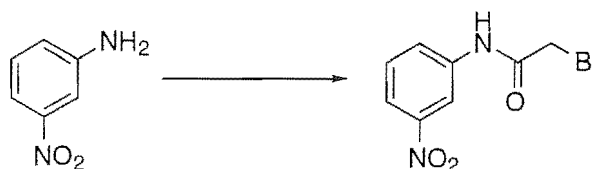
m.p. 200-203°C.

^1H NMR (CDCl_3) δ 4.25 (s, 2H, CH_2), 7.55 (t, 1H, $J=8.3\text{Hz}$, aryl CH), 7.96 (d, 1H, $J=8.3\text{Hz}$, aryl CH), 8.03 (d, 1H, $J=8.3\text{Hz}$, aryl CH), 8.46 (s, 1H, aryl CH), 8.56 (br.s, 1H, NH).

m/z (EI) 214.0 (M^+), 165.0, 138.0, 92.1.

High Res. $\text{C}_8\text{H}_7\text{N}_2\text{O}_3\text{Cl}$ calc. 214.01452; found 214.01460.

Preparation of *N*-(3-nitrophenyl) bromoacetamide 5.6



3-Nitroaniline (6g, 43.4mmol.) was dissolved in ethyl acetate (90mL). Bromoacetyl bromide (8.76g, 3.78mL, 43.3mmol.) was added by syringe and the resulting solution refluxed on an oil bath for 4 hours. The ethyl acetate was removed under reduced pressure to give *N*-(3-nitrophenyl) bromoacetamide as brown crystals (11.24g, 100%).

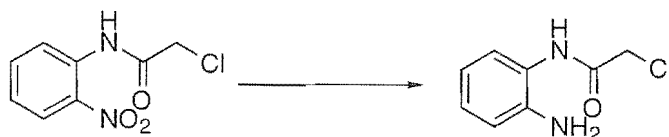
m.p. 68-69°C.

^1H NMR (CDCl_3) δ 4.08 (s, 2H, CH_2), 7.55 (t, 1H, $J=8.3\text{Hz}$, aryl CH), 7.96 (d, 1H, $J=8.3\text{Hz}$, aryl CH), 8.03 (d, 1H, $J=8.3\text{Hz}$, aryl CH), 8.43 (br.s, 2H, NH & aryl CH).

m/z (EI) 260.0 (M^+), 138.0, 92.1.

High Res. $\text{C}_8\text{H}_7\text{N}_2\text{O}_3^8\text{Br}$ calc. 259.96209; found 259.96237.

High Res. $\text{C}_8\text{H}_7\text{N}_2\text{O}_3^{79}\text{Br}$ calc. 257.96400; found 257.96339.



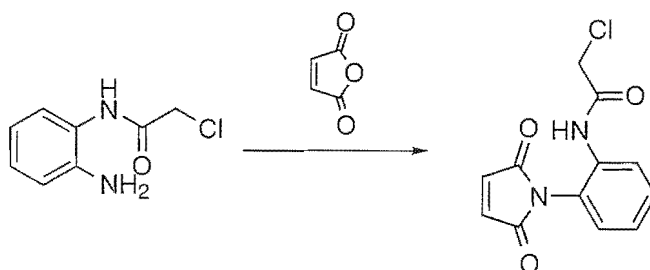
Preparation of *N*-(2-aminophenyl) chloroacetamide 5.7

N-(2-Nitrophenyl) chloroacetamide (2g, 9.32mmol.) was dissolved in ethyl acetate (20mL) and palladium on carbon (10%, 0.25g) added. Air was evacuated under reduced pressure and a hydrogen balloon added. Stirring under hydrogen was continued for 48 hours. The palladium on carbon was filtered out and washed with methanol. The methanol was removed under reduced pressure to give an oil. Purification by radial silica chromatography gave *N*-(2-aminophenyl) chloroacetamide as a pale yellow oil (586mg, 34%).

¹H NMR (CD₃CO₂D) δ 4.34 (s, 2H, CH₂), 7.31-7.52 (m, 4H, aryl CH).

m/z (EI) 184 (M⁺), 150, 108.

Preparation of *N*-2-(chloroacetamido)phenyl maleimide 5.8



N-(2-Aminophenyl) chloroacetamide (586mg, 3.17mmol.) was dissolved in dry THF (3mL) and maleic anhydride (311mg, 3.17mmol.) in dry THF (3mL) was added. The solution was stirred overnight resulting in a pale yellow precipitate which was filtered and placed in a conical flask with sodium acetate (100mg, 1.2mmol.). Acetic anhydride (25mL, 265mmol.) was added and the resulting suspension was dissolved by heating on a steam bath and then heated for a further 30 minutes. After cooling to room temperature the solution was partitioned between

ethyl acetate and water. The ethyl acetate layers were combined, dried (MgSO_4), and the ethyl acetate removed under reduced pressure to give a yellow oil. Purification by radial silica chromatography gave *N*-2-(chloroacetamido)phenyl maleimide as a pale yellow crystals (82.8mg, 10%).

m.p. 114-115°C.

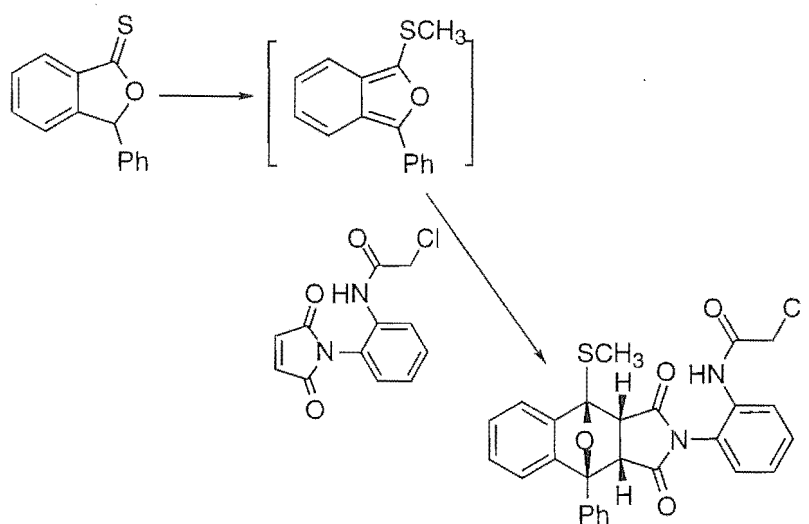
Analysis $\text{C}_{12}\text{H}_9\text{N}_2\text{O}_3\text{Cl}$ calc. C, 54.45; H, 3.43; N, 10.59; found C, 54.74; H, 3.44; N, 10.48.

^1H NMR (CDCl_3) δ 4.15 (s, 2H, CH_2), 6.94 (s, 2H, COCH), 7.25 (d, 1H, $J=8.3\text{Hz}$, aryl CH), 7.34 (t, 1H, $J=8.3\text{Hz}$, aryl CH), 7.48 (t, 1H, $J=8.3\text{Hz}$, aryl CH), 7.93 (d, 1H, $J=8.3\text{Hz}$, aryl CH), 8.40 (br. s, 1H, NH).

m/z (EI) 264 (M^+), 229 ($\text{M}-\text{Cl}^+$).

High Res. $\text{C}_{12}\text{H}_9\text{N}_2\text{O}_3\text{Cl}$ calc. 264.03019; found 264.03027.

Preparation of 9-endo-13-endo-1-methylsulfanyl-8-phenyl-11-(2-chloroacetamidophenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione 5.9



n-Butyl lithium (0.46mL of a 1.6M solution in hexane, 0.74mmol.) was added dropwise to a stirred solution of diisopropylamine (0.10mL, 0.74mmol.) in THF at

0°C under dry nitrogen. The solution was stirred for ten minutes at 0°C and then cooled to -78°C. A solution of 3-phenyl-1(3H)-isobenzofuranthione (151mg, 0.67mmol.) in THF was added and the mixture was stirred for 1 hour at -78°C. Methyl iodide (0.046mL, 0.74mmol.) was added dropwise to the solution, which was then allowed to warm to room temperature over a period of 2 hours. *N*-2-(Chloroacetamido)phenyl maleimide (177mg, 0.67mmol.) in THF was added and the reaction was stirred overnight under a drying tube. The solvent was removed under reduced pressure to give an oil which was partitioned between ethyl acetate and water. The organic layer was dried (MgSO₄) and the solvent removed under reduced pressure. This was purified by radial silica chromatography to give 9-*endo*-13-*endo*-1-methylsulfanyl-8-phenyl-11-(2-chloroacetamidophenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione as white crystals (50mg, 15%).

m.p. 162-163°C.

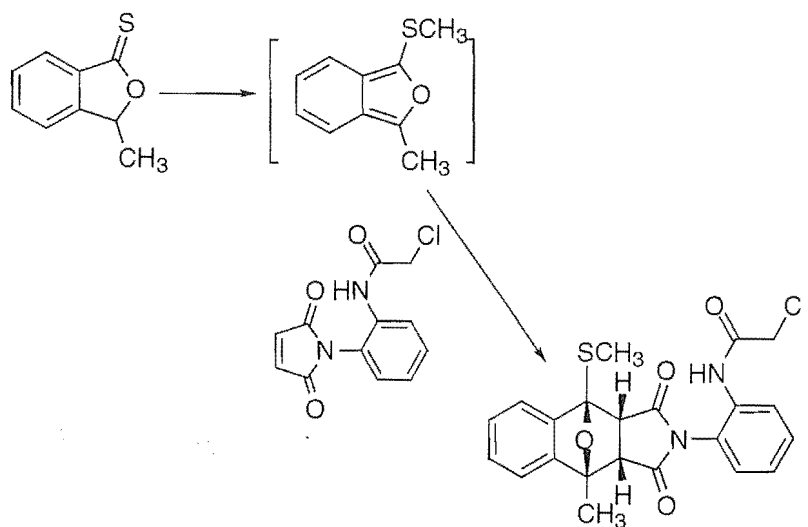
Analysis C₂₇H₂₁N₂SO₄Cl calc. C, 64.21; H, 4.19; N, 5.55; found C, 64.19; H, 4.50; N, 5.57.

¹H NMR (CDCl₃) δ 2.33 (s, 3H, SCH₃), 3.73 (s, 2H, CH₂Cl), 4.04 (d, 1H, J=8.3Hz, COCH), 4.27 (d, 1H, J=8.8Hz, COCH), 5.76 (d, 1H, J=7.8Hz, aryl CH), 6.99-7.11 (m, 2H, aryl CH), 7.26-7.56 (m, 7H, aryl CH), 7.62 (m, 1H, aryl CH), 7.91 (d, 2H, J=6.8Hz, aryl CH).

m/z (EI/ FAB) 520.1 (M⁺), 473.1 (M-CCl⁺), 256.0, 241.0.

High Res. *m/z* C₂₇H₂₁N₂SO₄Cl calc. 520.06822; found 520.06905.

Preparation of 9-endo-13-endo-1-methylsulfanyl-8-methyl-11-(2-chloroacetamidophenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione 5.10



n-Butyl lithium (0.19mL of a 1.6M solution in hexane, 0.301mmol.) was added dropwise to a stirred solution of diisopropylamine (0.04mL, 0.301mmol.) in THF at 0°C under dry nitrogen. The solution was stirred for ten minutes at 0°C and then cooled to -78°C. A solution of 3-methyl-1(3H)-isobenzofuranthione (45mg, 0.274mmol.) in THF was added and the mixture was stirred for 1 hour at -78°C. Methyl iodide (0.02mL, 0.330mmol.) was added dropwise to the solution, which was then allowed to warm to room temperature over a period of 2 hours. *N*-2-(Chloroacetamido)phenyl maleimide (73mg, 0.274mmol.) in THF was added and the reaction was stirred overnight under a drying tube. The solvent was removed under reduced pressure to give an oil which was partitioned between ethyl acetate and water. The organic layer was dried (MgSO₄) and the solvent removed under reduced pressure. This was purified by radial silica chromatography to give 9-endo-13-endo-1-methylsulfanyl-8-methyl-11-(2-chloroacetamidophenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione as white crystals (17mg, 14%).

m.p. 173-174°C.

Analysis $C_{22}H_{19}N_2SO_4Cl$ calc. C, 59.66; H, 4.32; N, 6.33; found C, 60.03, H, 3.99; N, 6.11.

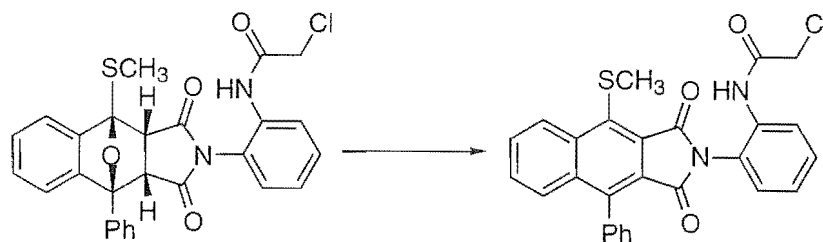
1H NMR ($CDCl_3$) δ 2.02 (s, 3H, CH_3), 2.18 (s, 3H, SCH_3), 3.10 (d, 1H, $J=6.4Hz$, COCH), 3.23 (d, 1H, $J=6.4Hz$, COCH), 3.83 (s, 2H, CH_2), 7.18-7.27 (m, 2H, aryl CH), 7.34-7.49 (m, 4H, aryl CH), 7.64 (s, 1H, aryl CH), 8.33 (d, 1H, $J=8.3Hz$, aryl CH).

m/z (EI) 442 (M^+), 395 ($M-CCl^+$), 163.

High Res. m/z $C_{21}H_{19}N_2SO_4$ calc. 395.10662; found 395.10614.

IR ($CDCl_3$) ν_{max} 1720.4, 1602.7, 1458.1, 1382.9, 1236.3 cm^{-1} .

Preparation of *N*-(2-chloroacetamidophenyl) 1-methylsulfanyl-8-phenyl-naphthalene-2,3-dicarboximide 5.11



9-endo-13-endo-1-Methylsulfanyl-8-phenyl-11-(2-chloroacetamidophenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione (10mg, 0.02mmol.) and trifluoroacetic acid (1mL, 13mmol.) were stirred together in dichloromethane (5mL) for 18 hours. The dichloromethane and excess trifluoroacetic acid were removed under reduced pressure to give an oil which was purified by radial silica chromatography to give *N*-(2-chloroacetamidophenyl) 1-methylsulfanyl-8-phenyl-naphthalene-2,3-dicarboximide as pale brown crystals (1.4mg, 14%).

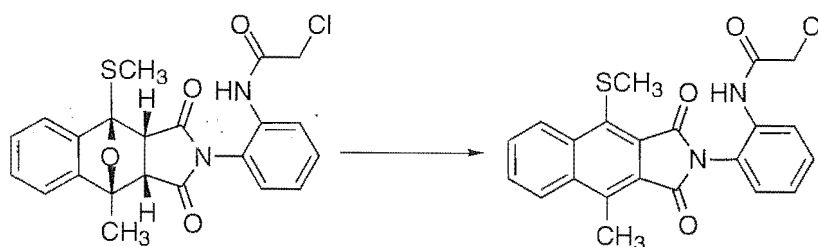
m.p. 170°C (decomp.).

^1H NMR (CDCl_3) δ 2.70 (s, 3H, SCH_3), 3.69 (s, 2H, CH_2Cl), 7.24-7.44 (m, 6H, aryl CH), 7.51 (m, 2H, aryl CH), 7.64 (t, 1H, $J=6.8\text{Hz}$, aryl CH), 7.75-7.83 (m, 2H, aryl CH), 8.06 (m, 1H, aryl CH), 9.07 (d, 1H, $J=9.8\text{Hz}$, aryl CH).

m/z (EI) 486 (M^+), 451 ($\text{M}-\text{Cl}^+$), 436 ($\text{M}-\text{CH}_3\text{Cl}^+$), 410 ($\text{M}-\text{COCHCl}^+$).

High Res. m/z $\text{C}_{26}\text{H}_{16}\text{N}_2\text{SO}_3$ calc. 436.08817; found 436.08841.

Preparation of *N*-(2-chloroacetamidophenyl) 1-methylsulfanyl-8-methyl-naphthalene-2,3-dicarboximide 5.12



9-endo-13-endo-1-Methylsulfanyl-8-methyl-11-(2-chloroacetamidophenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione (12mg, 0.027mmol.) and trifluoroacetic acid (1mL, 13mmol.) were stirred together in dichloromethane (5mL) for 18 hours. The dichloromethane and excess trifluoroacetic acid were removed under reduced pressure to give an oil which was purified by radial silica chromatography to give *N*-(2-chloroacetamidophenyl) 1-methylsulfanyl-8-methyl-naphthalene-2,3-dicarboximide as a pale brown solid (3mg, 26%).

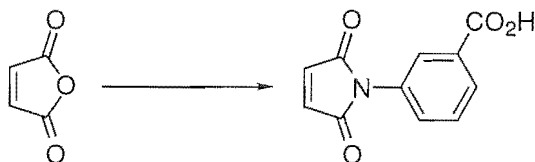
m.p. 180°C (decomp.).

^1H NMR (CDCl_3) δ 2.62 (s, 3H, SCH_3), 3.16 (s, 3H, CH_3), 3.75 (s, 2H, CH_2Cl), 7.38 (t, 2H, $J=7.8\text{Hz}$, aryl CH), 7.49 (t, 1H, $J=8.3\text{Hz}$, aryl CH), 7.79-7.88 (m, 3H, aryl CH), 8.08 (br.s, 1H, NH), 8.30 (d, 1H, $J=6.8\text{Hz}$, aryl CH), 9.04 (d, 1H, $J=6.9\text{Hz}$, aryl CH).

m/z (EI) 424 (M^+), 389 ($\text{M}-\text{Cl}^+$), 374 ($\text{M}-\text{CH}_3\text{Cl}^+$), 348 ($\text{M}-\text{COCHCl}^+$).

High Res. m/z $C_{22}H_{17}N_2SO_3$ calc. 389.09599; found 389.09538.

Preparation of *N*-(3-carboxyphenyl) maleimide 5.13



3-Aminobenzoic acid (10.0g, 73mmol.) was dissolved in dry THF (30mL) and maleic anhydride (7.15g, 73mmol.) in dry THF (10mL) was added. The solution was stirred for 1 hour resulting in a yellow precipitate which was filtered and then mixed with sodium acetate (2g, 24mmol.) and acetic anhydride (200mL, 2.12mol.). The resulting suspension was heated on a steam bath until all had dissolved and then for a further 30 minutes. After cooling to room temperature the solution was partitioned between ethyl acetate and water. The ethyl acetate layers were combined, dried ($MgSO_4$), and the ethyl acetate removed under reduced pressure to give *N*-(3-carboxyphenyl) maleimide as yellow crystals (14.27g, 90%).

m.p. 223-225°C.

Analysis $C_{11}H_7NO_4$ calc. C, 60.83; H, 3.25; N, 6.45; found C, 61.15; H, 3.10; N, 6.46.

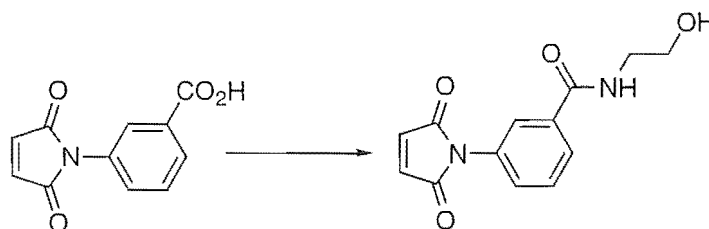
1H NMR ($CDCl_3$) δ 6.98 (s, 2H, COCH), 7.56 (m, 2H, aryl CH), 8.00 (m, 2H, aryl CH).

m/z (EI) 217.0 (M^+), 173.0 ($M-CO_2^+$).

High Res. m/z $C_{11}H_7NO_4$ calc. 217.03751; found 217.03762.

IR ($CDCl_3$) ν_{max} 1720.4, 1591.2, 1454.2, 1384.8, 1234.4, 1147.6 cm^{-1} .

**Preparation of *N*-(3-(2-aza-4-hydroxy-1-oxobutanyl)phenyl)
maleimide 5.14**



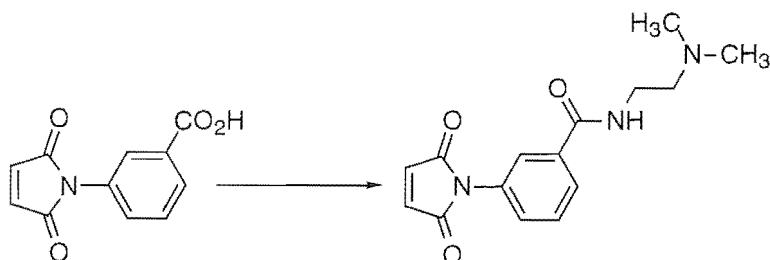
N-(3-Carboxyphenyl) maleimide (1g, 4.6mmol.) was stirred in dichloromethane (5mL) and DMF was added dropwise until dissolution was complete. DCC (950mg, 4.6mmol.) in dichloromethane (1mL), HOBt (622mg, 4.6mmol.) in DMF (1mL), and ethanolamine (0.28mL, 4.6mmol.) in dichloromethane (1mL) were added in the given order and the solution stirred for 6 hours. The resulting precipitate was filtered and the solvent removed under reduced pressure. The product from the supernatant was partitioned between water and ethyl acetate, the organic fractions were combined, dried (MgSO₄), and evaporated to give *N*-(3-(2-aza-4-hydroxy-1-oxobutanyl)phenyl) maleimide as a pale yellow solid (295mg, 25%).

m.p. 105-106°C (decomp.).

¹H NMR (CDCl₃/ CD₃OD) δ 3.46 (m, 2H, NHCH₂), 3.63 (m, 2H, CH₂OH), 6.87 (s, 2H, COCH), 7.51 (m, 2H, aryl CH), 7.77 (m, 2H, aryl CH).

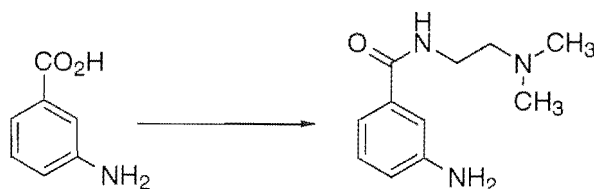
m/z (EI) 260 (M⁺), 172.

**Preparation of *N*-(3-(2-aza-4-dimethylamino-1-oxobutanyl)phenyl)
maleimide 5.15**



N-(3-Carboxyphenyl) maleimide (500mg, 2.3mmol.) was stirred in dichloromethane (5mL) and DMF was added dropwise until dissolved. DCC (475mg, 2.3mmol.) in dichloromethane (3mL), HOBT (311mg, 2.3mmol.) in DMF (3mL), and *N,N*-dimethylethylenediamine (0.25mL, 2.3mmol.) in dichloromethane (1mL) were added in the given order and the solution stirred for 18 hours. The resulting precipitate was removed by filtration. The supernatant was evaporated and the residue was partitioned between hydrochloric acid (1M, 10mL) and ethyl acetate. The aqueous phase was neutralised with sodium bicarbonate solution until basic (pH paper), and extracted with ethyl acetate (10mL). The organic fractions from the basic solution were combined, dried (MgSO₄), and evaporated to give *N*-(3-(2-aza-4-dimethylamino-1-oxobutanyl) phenyl)maleimide as a red solid (30mg, 5%).

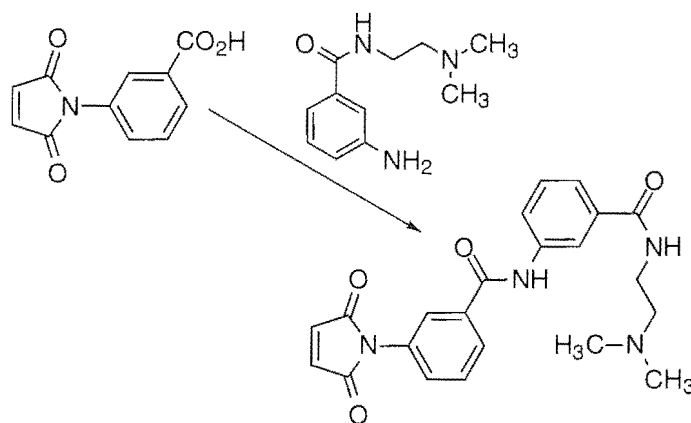
¹H NMR (CDCl₃/ CD₃OD) δ 2.27 (s, 6H, N(CH₃)₂), 2.54 (t, 2H, CH₂N), 3.52 (m, 2H, NHCH₂), 6.84 (s, 2H, COCH), 7.46-7.51 (m, 2H, aryl CH), 7.78 (m, 2H, aryl CH).

Preparation of 3-(2-aza-4-dimethylamino-1-oxobutanyl)aniline**5.17**

3-Aminobenzoic acid (1.00g, 7.3mmol.) was stirred in dichloromethane (10mL) and DMF was added dropwise until dissolved. DCC (1.56g, 7.3mmol.) in dichloromethane (5mL), HOBT (986mg, 7.3mmol.) in DMF (5mL), and *N,N*-dimethylethylenediamine (0.800mL, 7.3mmol.) in dichloromethane (2mL) were added in the given order and the solution stirred for 16 hours. The resulting precipitate was filtered. The supernatant was evaporated and the residue was partitioned between hydrochloric acid (1M, 20mL) and ethyl acetate (20mL). The aqueous layer was neutralised with sodium hydroxide solution (1M, 30mL) and extracted with ethyl acetate. The organic fractions were combined, dried (MgSO₄), and evaporated to give 3-(2-aza-4-dimethylamino-1-oxobutanyl)aniline as a yellow oil (433mg, 29%).

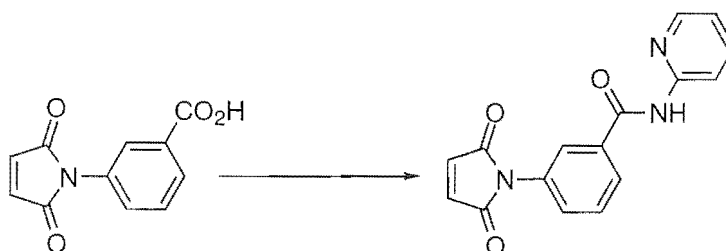
¹H NMR (CDCl₃/ CD₃OD) δ 2.26 (s, 6H, N(CH₃)₂), 2.52 (m, 2H, CH₂), 3.49 (m, 2H, CH₂), 6.74 (m, 1H, aryl CH), 6.95 (br.s, 1H, CONH), 7.05-7.16 (m, 3H, aryl CH).

Preparation of *N*-3-(3-*N*-(*N,N*-dimethylaminoethyl)carboxamidophenyl)carboxamidophenyl maleimide 5.18



N-(3-Carboxyphenyl) maleimide (450mg, 2.1mmol.) was stirred in dichloromethane (5mL) and DMF was added dropwise until dissolved. DCC (428mg, 2.1mmol.) in dichloromethane (3mL), HOBt (280mg, 2.1mmol.) in DMF (3mL), and 3-(2-aza-4-dimethylamino-1-oxobutanyl)aniline (430mg, 2.1mmol.) in dichloromethane (1mL) were added in the given order and the solution stirred for 18 hours. The resulting precipitate was removed by filtration. The supernatant was evaporated and the residue was partitioned between hydrochloric acid (1M, 10mL) and ethyl acetate (10mL). The aqueous layer neutralised with sodium bicarbonate solution until basic to pH paper, and extracted with ethyl acetate. The latter organic fractions were combined, dried (MgSO₄), and evaporated to give *N*-3-(3-*N*-(*N,N*-dimethylaminoethyl)carboxamidophenyl)carboxamidophenyl maleimide as a red oil (20mg, 3%).

¹H NMR (CDCl₃/ CD₃OD) δ 2.44 (s, 6H, N(CH₃)₂), 2.75 (m, 2H, CH₂), 3.63 (m, 2H, CH₂), 6.85 (s, 2H, COCH), 7.40 (d, 1H, J=8.3Hz, aryl CH), 7.52-7.63 (m, 3H, aryl CH), 7.91-7.99 (m, 3H, aryl CH), 8.80 (br.s, 1H, CONH).

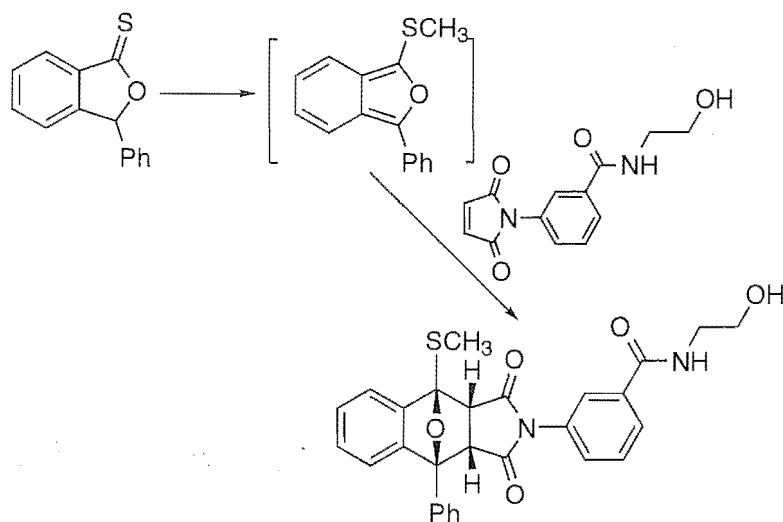
Preparation of *N*-3-(*N*-(2-pyridyl))carboxamidophenyl maleimide**5.19**

N-(3-Carboxyphenyl) maleimide (500mg, 2.3mmol.) was stirred in dichloromethane (5mL) and DMF was added dropwise until dissolved. DCC (474mg, 2.3mmol.) in dichloromethane (3mL), HOBT (310mg, 2.3mmol.) in DMF (3mL), and 2-aminopyridine (216mg, 2.3mmol.) in a mixture of dichloromethane (3mL) and DMF (3mL) were added in the given order and the solution was stirred for 6 hours, during which time a white solid precipitated. The solution was filtered and the supernatant was evaporated. The product was partitioned between hydrochloric acid solution (1M, 20mL) and ethyl acetate. The aqueous layer was neutralised with sodium hydroxide solution (1M, 30mL) and extracted with ethyl acetate. The organic fractions were combined, washed with water, dried (MgSO₄), and evaporated to give *N*-3-(*N*-(2-pyridyl))carboxamidophenyl maleimide as a brown oil (246mg, 36%).

¹H NMR (CDCl₃/ CD₃OD) δ 6.93 (s, 2H, COCH), 7.40-7.57 (m, 4H, aryl CH), 7.74 (d, 1H, *J*=7.8Hz, aryl CH), 7.84 (d, 1H, *J*=8.3Hz, aryl CH), 8.06 (m, 2H, aryl CH).

m/z (EI) 293 (M⁺), 216 (M-C₅H₃N⁺).

Preparation of 9-*endo*-13-*endo*-1-methylsulfanyl-8-phenyl-11-(3-(2-aza-4-hydroxy-1-oxobutanyl)phenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione 5.20



n-Butyl lithium (0.76mL of a 1.6M solution in hexane, 1.2mmol.) was added dropwise to a stirred solution of diisopropylamine (0.17mL, 1.2mmol.) in THF at 0°C under dry nitrogen. The solution was stirred for ten minutes at 0°C and then cooled to -78°C. A solution of 3-phenyl-1(3H)-isobenzofuranthione (230mg, 1.0mmol.) in THF was added and the mixture was stirred for 1 hour at -78°C. Methyl iodide (0.076mL, 1.2mmol.) was added dropwise to the solution, which was then allowed to warm to room temperature over a period of 2 hours. *N*-(3-(2-Aza-4-hydroxy-1-oxobutanyl)phenyl) maleimide (319mg, 1.2mmol.) in THF with a drop of methanol was added and the reaction was stirred overnight under a drying tube. The solvent was removed under reduced pressure to give an oil which was partitioned between ethyl acetate and water. The organic layer was dried (MgSO₄) and the solvent removed under reduced pressure. This was purified by radial silica chromatography to give 9-*endo*-13-*endo*-1-methylsulfanyl-8-phenyl-11-(3-(2-aza-4-hydroxy-1-oxobutanyl)phenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione as crystals (85mg, 18%).

m.p. 154-156°C.

Analysis $C_{28}H_{24}N_2SO_5$ calc. C, 67.18; H, 4.83; N, 5.60; found C, 67.32, H, 4.80, N, 5.60.

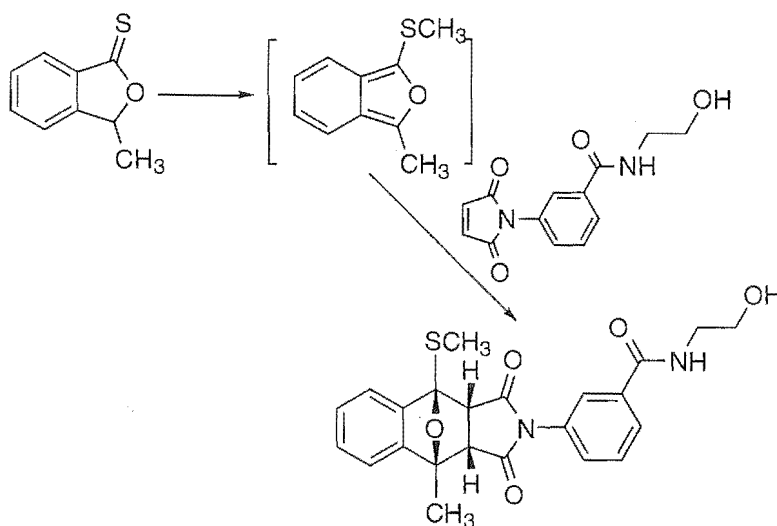
1H NMR ($CDCl_3$) δ 2.37 (s, 3H, SCH₃), 3.52 (m, 2H, NHCH₂), 3.73 (m, 2H, CH₂OH), 4.01 (d, 1H, J=8.3Hz, COCH), 4.27 (d, 1H, J=8.7Hz, COCH), 6.55 (d, 1H, J=7.8Hz, aryl CH), 6.95 (s, 1H, aryl CH), 7.05 (d, 1H, J=7.3Hz, aryl CH), 7.34-7.54 (m, 6H, aryl CH), 7.65 (m, 1H, aryl CH), 7.74 (d, 1H, J=8.3Hz, aryl CH), 7.93 (d, 2H, J=8.3Hz, aryl CH).

m/z (EI) 500 (M^+), 482 ($M-H_2O^+$), 260, 240.

High Res. $C_{28}H_{24}N_2SO_5$ m/z calc. 500.14068; found 500.1482.

IR ($CDCl_3/CD_3OD$) ν_{max}/cm^{-1} 1718.5, 1660.6, 1521.7, 1458.1, 1377.1, 1344.3, 1276.8, 1184.2.

Preparation of 9-endo-13-endo-1-methylsulfanyl-8-methyl-11-(3-(2-aza-4-hydroxy-1-oxobutanyl)phenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione 5.21



n-Butyl lithium (0.54mL of a 1.6M solution in hexane, 0.87mmol.) was added dropwise to a stirred solution of diisopropylamine (0.12mL, 0.87mmol.) in THF at

0°C under dry nitrogen. The solution was stirred for ten minutes at 0°C and then cooled to -78°C. A solution of 3-methyl-1(3H)-isobenzofuranthione (130mg, 0.79mmol.) in THF was added and the mixture was stirred for 1 hour at -78°C. Methyl iodide (0.054mL, 0.87mmol.) was added dropwise to the solution, which was then allowed to warm to room temperature over a period of 2 hours. *N*-(3-(2-Aza-4-hydroxy-1-oxobutanyl)phenyl) maleimide (206mg, 0.79mmol.) in THF with a drop of methanol was added and the reaction was stirred overnight under a drying tube. The solvent was removed under reduced pressure to give an oil which was partitioned between ethyl acetate and water. The organic layer was dried (MgSO₄) and the solvent removed under reduced pressure. This was purified by radial silica chromatography to give 9-*endo*-13-*endo*-1-methylsulfanyl-8-methyl-11-(3-(2-aza-4-hydroxy-1-oxobutanyl)phenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione as yellow crystals (15mg, 4%).

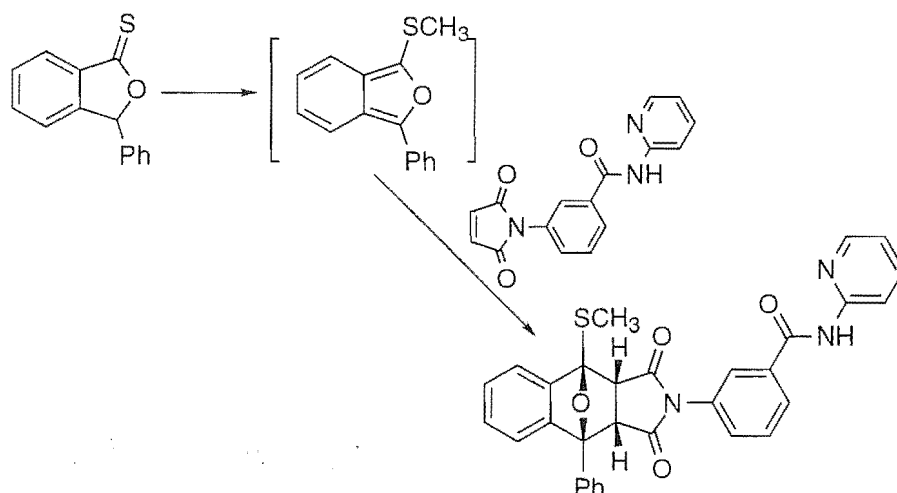
m.p. 127-129°C.

¹H NMR (CDCl₃/ CD₃OD) δ 2.06 (s, 3H, CH₃), 2.14 (s, 3H, SCH₃), 3.08 (d, 1H, J=6.8Hz, COCH), 3.21 (d, 1H, J=6.8Hz, COCH), 3.47 (m, 2H, NHCH₂), 3.86 (m, 2H, CH₂OH), 7.30-7.50 (m, 5H, aryl CH), 7.75 (s, 1H, aryl CH), 7.83 (d, 1H, J=7.8Hz, aryl CH), 7.93 (m, 1H, aryl CH).

m/z (EI) 438 (M⁺), 420 (M-H₂O⁺), 260, 178.

High Res. C₂₃H₂₂N₂SO₅ *m/z* calc. 438.12502; found 438.12505.

Preparation of 9-endo-13-endo-1-methylsulfanyl-8-phenyl-11-(3-(*N*-(2-pyridyl))carboxamidophenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}] tetradeca-2,4,6-triene-10,12-dione 5.24



n-Butyl lithium (0.69mL of a 1.6M solution in hexane, 1.1mmol.) was added dropwise to a stirred solution of diisopropylamine (0.16mL, 1.1mmol.) in THF at 0°C under dry nitrogen. The solution was stirred for ten minutes at 0°C and then cooled to -78°C. A solution of 3-phenyl-1(3H)-isobenzofuranthione (250mg, 1.1mmol.) in THF was added and the mixture was stirred for 1 hour at -78°C. Methyl iodide (0.07mL, 1.1mmol.) was added dropwise to the solution, which was then allowed to warm to room temperature over a period of 2 hours. *N*-3-(*N*-(2-Pyridyl))carboxamidophenyl maleimide (310mg, 1.1mmol.) in THF with a drop of methanol was added and the reaction was stirred overnight under a drying tube. The solvent was removed under reduced pressure to give an oil which was partitioned between ethyl acetate and water. The organic layer was dried (MgSO₄) and the solvent removed under reduced pressure. This was purified by radial silica chromatography to give 9-endo-13-endo-1-methylsulfanyl-8-phenyl-11-(3-(*N*-(2-pyridyl))carboxamidophenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}] tetradeca-2,4,6-triene-10,12-dione as an oil (6mg, 1%).

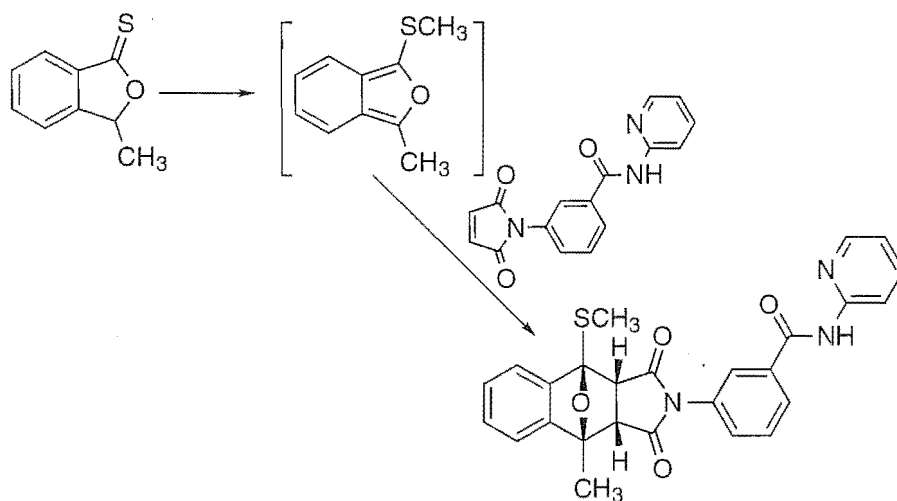
^1H NMR ($\text{CDCl}_3/\text{CD}_3\text{OD}$) δ 2.35 (s, 3H, SCH₃), 3.96 (d, 1H, $J=8.3\text{Hz}$, COCH), 4.20 (d, 1H, $J=8.3\text{Hz}$, COCH), 7.29-7.62 (m, 9H, aryl CH), 7.74 (t, 2H, $J=7.8\text{Hz}$, aryl CH), 7.85 (d, 2H, $J=8.3\text{Hz}$, aryl CH), 7.89-7.94 (m, 3H, aryl CH), 8.01 (d, 1H, $J=7.8\text{Hz}$, aryl CH).

m/z (EI) 533 (M^+), 457 ($\text{M}-\text{C}_5\text{H}_2\text{N}^+$), 240.

High Res. $\text{C}_{26}\text{H}_{21}\text{N}_2\text{SO}_4$ m/z calc. 457.12228; found 457.12260.

IR ($\text{CDCl}_3/\text{CD}_3\text{OD}$) ν_{max} 1793.7, 1720.4, 1286.4, 1234.4 cm^{-1} .

Preparation of 9-endo-13-endo-1-methylsulfanyl-8-methyl-11-(3-(*N*-(2-pyridyl))carboxamidophenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}] tetradeca-2,4,6-triene-10,12-dione 5.25



n-Butyl lithium (0.19mL of a 1.6M solution in hexane, 0.30mmol.) was added dropwise to a stirred solution of diisopropylamine (0.04mL, 0.30mmol.) in THF at 0°C under dry nitrogen. The solution was stirred for ten minutes at 0°C and then cooled to -78°C. A solution of 3-methyl-1(3H)-isobenzofuranthione (45mg, 0.27mmol.) in THF was added and the mixture was stirred for 1 hour at -78°C. Methyl iodide (0.02mL, 0.33mmol.) was added dropwise to the solution, which was then allowed to warm to room temperature over a period of 2 hours. *N*-3-(*N*-

(2-Pyridyl))carboxamidophenyl maleimide (73mg, 0.27mmol.) in THF with a drop of methanol was added and the reaction was stirred overnight under a drying tube. The solvent was removed under reduced pressure to give an oil which was partitioned between ethyl acetate and water. The organic layer was dried (MgSO₄) and the solvent removed under reduced pressure. This was purified by radial silica chromatography to give 9-*endo*-13-*endo*-1-methylsulfanyl-8-methyl-11-(3-(*N*-(2-pyridyl))carboxamidophenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}] tetradeca-2,4,6-triene-10,12-dione as an oil (8mg, 6%).

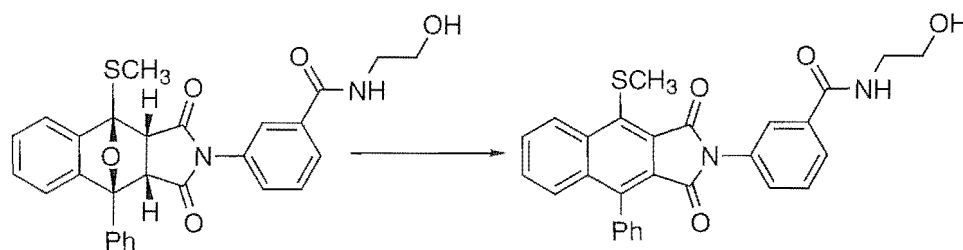
¹H NMR (CDCl₃/ CD₃OD) δ 2.25 (s, 3H, CH₃), 2.52 (s, 3H, SCH₃), 3.67 (d, 1H, J=8.3Hz, COCH), 3.82 (d, 1H, J=8.3Hz, COCH), 6.61 (d, 1H, J=8.4Hz, aryl CH), 6.87 (m, 1H, aryl CH), 7.05-7.40 (m, 6H, aryl CH), 7.54 (m, 2H, aryl CH), 7.91 (d, 1H, J=8.3Hz, aryl CH), 8.02 (m, 1H, aryl CH).

m/z (EI) 471.5 (M⁺), 378.1 (M-C₅H₅N₂⁺).

High Res. C₂₁H₁₆NSO₄ *m/z* calc. 378.08001; found 378.08026.

IR (CDCl₃/ CD₃OD) ν_{\max} 2933.5, 1720.4, 1380.9, 1278.7, 1236.3 cm⁻¹.

Preparation of *N*-3-(2-aza-4-hydroxy-1-oxobutanyl)phenyl 1-methylsulfanyl-8-phenyl-naphthalene-2,3-dicarboximide 5.27



9-*endo*-13-*endo*-1-Methylsulfanyl-8-phenyl-11-(3-(2-aza-4-hydroxy-1-oxobutanyl)phenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione (30mg, 0.062mmol.) and trifluoroacetic acid (1mL, 13mmol.) were stirred together in dichloromethane (3mL) for 48 hours. The dichloromethane and excess trifluoroacetic acid were removed under reduced pressure to give an oil

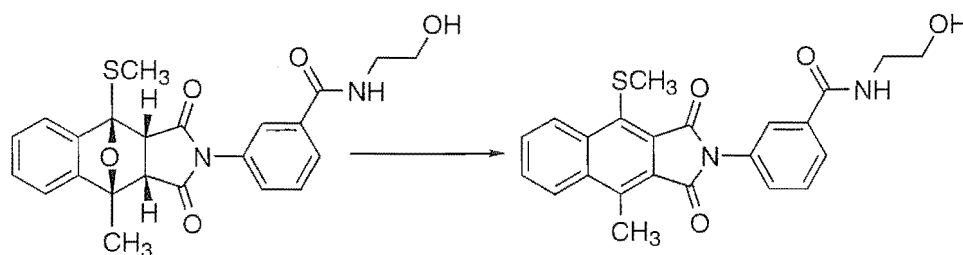
which was purified by radial silica chromatography to give *N*-3-(2-aza-4-hydroxy-1-oxobutanyl)phenyl 1-methylsulfanyl-8-phenyl-naphthalene-2,3-dicarboximide as an oil (5mg, 17%).

^1H NMR ($\text{CDCl}_3/\text{CD}_3\text{OD}$) δ 2.47 (s, 3H, SCH₃), 3.62 (m, 2H, NHCH₂), 4.40 (t, 2H, J=6.8Hz, CH₂OH), 6.93 (d, 1H, J=7.3Hz, aryl CH), 7.09 (m, 4H, aryl CH), 7.26 (s, 1H, aryl CH), 7.32-7.47 (m, 4H, aryl CH), 7.65 (d, 2H, J=7.8Hz, aryl CH), 8.01 (d, 1H, J=7.8Hz, aryl CH).

m/z (EI) 482 (M^+).

High Res. $\text{C}_{28}\text{H}_{22}\text{N}_2\text{SO}_4$ m/z calc. 482.13011; found 482.1283.

Preparation of *N*-3-(2-aza-4-hydroxy-1-oxobutanyl)phenyl 1-methylsulfanyl-8-methyl-naphthalene-2,3-dicarboximide 5.28



9-*endo*-13-*endo*-1-Methylsulfanyl-8-methyl-11-(3-(2-aza-4-hydroxy-1-oxobutanyl)phenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione (5.0mg, 0.011mmol.) and trifluoroacetic acid (0.5mL, 6.5mmol.) were stirred together in dichloromethane (5mL) for 18 hours. The dichloromethane and excess trifluoroacetic acid were removed under reduced pressure to give an oil which was purified by radial silica chromatography to give *N*-3-(2-aza-4-hydroxy-1-oxobutanyl)phenyl 1-methylsulfanyl-8-methyl-naphthalene-2,3-dicarboximide as a yellow oil (1.2mg, 25%).

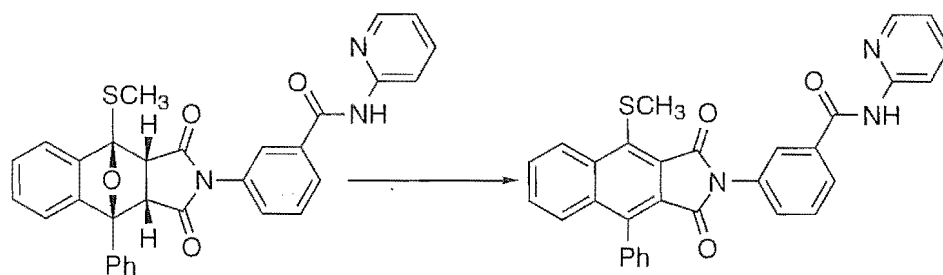
^1H NMR (CDCl_3) δ 2.56 (s, 3H, CH₃), 3.10 (s, 3H, SCH₃), 3.62 (m, 2H, NHCH₂), 3.82 (m, 2H, CH₂OH), 6.98 (br. s, 1H, NH), 7.51-7.63 (m, 2H, aryl

CH), 7.74-7.84 (m, 3H, aryl CH), 7.90 (s, 1H, aryl CH), 8.23 (d, 1H, $J=7.3\text{Hz}$, aryl CH), 8.97 (d, 1H, $J=8.3\text{Hz}$, aryl CH).

m/z (EI) 420 (M^+).

High Res. $\text{C}_{23}\text{H}_{20}\text{N}_2\text{SO}_4$ m/z calc. 420.11445; found 420.11468.

Preparation of *N*-3-(*N*-(2-pyridyl))carboxamidophenyl 1-methylsulfanyl-8-phenyl-naphthalene-2,3-dicarboximide 5.29



9-*endo*-13-*endo*-1-Methylsulfanyl-8-phenyl-11-(3-(*N*-(2-pyridyl))carboxamidophenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione (4mg, 0.0075mmol.) and trifluoroacetic acid (0.5mL, 6.5mmol.) were stirred together in dichloromethane (5mL) for 18 hours. The dichloromethane and excess trifluoroacetic acid were removed under reduced pressure to give an oil which was purified by radial silica chromatography to give *N*-3-(*N*-(2-pyridyl))carboxamidophenyl 1-methylsulfanyl-8-phenyl-naphthalene-2,3-dicarboximide as an oil (0.5mg, 13%).

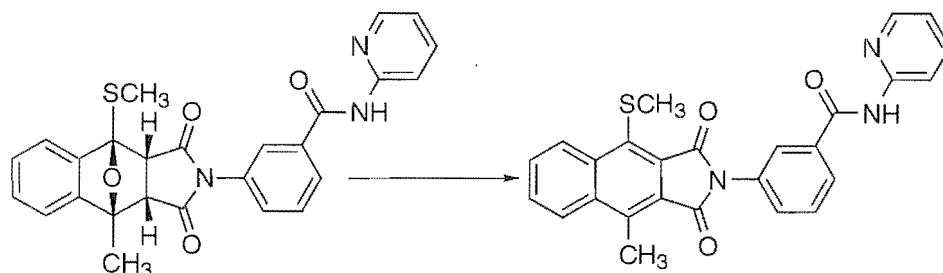
^1H NMR ($\text{CDCl}_3/\text{CD}_3\text{OD}$) δ 2.69 (s, 3H, SCH_3), 7.18-7.43 (m, 5H, aryl CH), 7.47-7.82 (m, 8H, aryl CH), 7.91-8.11 (m, 3H, aryl CH), 9.08 (d, 1H, $J=8.8\text{Hz}$, aryl CH).

m/z (EI) 515 (M^+), 439 ($M-\text{C}_5\text{H}_2\text{N}^+$), 422 ($M-\text{C}_5\text{H}_5\text{N}_2^+$).

High Res. $\text{C}_{26}\text{H}_{19}\text{N}_2\text{SO}_3$ m/z calc. 439.11171; found 439.11179.

IR ($\text{CDCl}_3/\text{CD}_3\text{OD}$) ν_{max} 1768.6, 1716.5, 1377.1, 1286.4, 1236.3 cm^{-1} .

Preparation of *N*-3-(*N*-(2-pyridyl))carboxamidophenyl 1-methylsulfanyl-8-methyl-naphthalene-2,3-dicarboximide 5.30



9-*endo*-13-*endo*-1-Methylsulfanyl-8-methyl-11-(3-(*N*-(2-pyridyl))carboxamidophenyl)-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}] tetradeca-2,4,6-triene-10,12-dione (5.5mg, 0.012mmol.) and trifluoroacetic acid (0.5mL, 6.5mmol.) were stirred together in dichloromethane (5mL) for 18 hours. The dichloromethane and excess trifluoroacetic acid were removed under reduced pressure to give an oil which was purified by radial silica chromatography to give *N*-3-(*N*-(2-pyridyl))carboxamidophenyl 1-methylsulfanyl-8-methyl-naphthalene-2,3-dicarboximide as an oil (1.0mg, 18%).

¹H NMR (CDCl₃/ CD₃OD) δ 2.56 (s, 3H, CH₃), 3.16 (s, 3H, SCH₃), 7.39-7.62 (m, 4H, aryl CH), 7.65-7.82 (m, 3H, aryl CH), 8.00-8.10 (m, 3H, aryl CH), 8.50 (d, 1H, J=8.3Hz, aryl CH), 9.01 (d, 1H, J=7.8Hz, aryl CH).

m/z (EI) 453 (M⁺), 391 (M-C₂H₆S⁺), 376 (M-C₅H₃N⁺).

High Res. C₂₆H₁₉N₃SO₃ *m/z* calc. 453.11475; found 453.11466.

IR (CDCl₃/ CD₃OD) ν_{max} 1764.7, 1712.7, 1490.4, 1450.4, 1379.0, 1276.8, 1164.9, 1132.1, 1107.1 cm⁻¹.

7.6 Chapter 6 Experimental

Preparation of 2-(benzylmercaptomethyl)benzoic acid 6.1⁷⁸

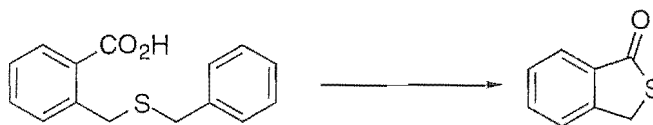


1(3H)-Isobenzofuranone (4.00g, 30mmol.) and sodium ethoxide (2.03g, 30mmol.) were added to benzyl mercaptan (3.70g, 30mmol.) and refluxed in ethanol (20mL) for 3 hours. The ethanol was removed under reduced pressure. Ice water (5mL) was added, acidified with concentrated sulfuric acid (5mL), and extracted with dichloromethane. The solution was concentrated under reduced pressure and crystallisation gave 2-(benzylmercaptomethyl)benzoic acid as white crystals (4.36g, 56%).

m.p. 84-85°C (lit. 84-85°C).

¹H NMR (CDCl₃) δ 3.66 (s, 2H, CH₂), 4.08 (s, 2H, CH₂), 7.22-7.32 (m, 6H, aryl CH), 7.35 (t, 1H, J= 7.8Hz, aryl CH), 7.48 (t, 1H, J=7.8Hz, aryl CH), 8.06 (d, 1H, J=7.8Hz, aryl CH).

Preparation of 1(3H)-isobenzothiophenone 6.2⁷⁸



2-Benzylmercaptomethyl benzoic acid (4.00g, 15.5mmol.) was added to polyphosphoric acid, formed by heating phosphorous pentoxide (27.78g, 98mmol.) with orthophosphoric acid (18.58mL, 98mmol.) at 100°C for 10 minutes, and stirred at 120°C for 30 minutes. After cooling ice water (20mL) was added and the solution extracted with chloroform, dried (Na₂SO₄), and the

chloroform removed under reduced pressure. Distillation under reduced pressure followed by recrystallisation from ethanol gave 1(3H)-isobenzothiophenone as white needles (1.25g, 54%).

m.p. 58-60°C (58-60°C).

^1H NMR (CDCl_3) δ 4.48 (s, 2H, CH_2), 7.48 (t, 1H, $J=7.8\text{Hz}$, aryl CH), 7.63 (t, 1H, $J=7.8\text{Hz}$, aryl CH), 7.84 (d, 1H, $J=7.4\text{Hz}$, aryl CH).

Preparation of 1(3H)-isobenzo[c]thiophene-1-thione 6.3⁷⁹



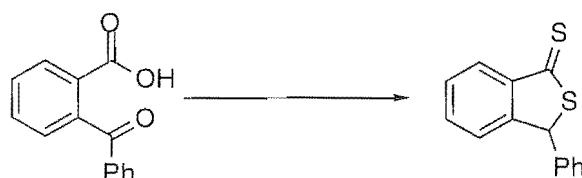
1,3-Dihydro-2-benzothiophen-1-one (700mg, 4.7mmol.) and Lawesson's reagent (943mg, 2.4mmol.) were refluxed in toluene (10mL) for 12 hours. The toluene was removed under reduced pressure, and the crude solid dissolved in ether and filtered to remove ether-insoluble components. Purification by radial silica chromatography gave 1(3H)-isobenzo[c]thiophene-1-thione as orange crystals (180mg, 23%).

m.p. 63-64°C (lit.63-64°C).

^1H NMR (CDCl_3) δ 4.53 (s, 2H, CH_2), 7.47 (t, 1H, $J=7.3\text{Hz}$, aryl CH), 7.54 (d, 1H, $J=6.4\text{Hz}$, aryl CH), 7.60 (t, 1H, $J=8.3\text{Hz}$, aryl CH), 8.07 (d, 1H, $J=7.8\text{Hz}$, aryl CH).

^{13}C NMR (CDCl_3) δ 40.8, 124.4, 125.4, 128.0, 132.6, 144.1, 147.1, 228.4.

IR ν_{max} (KBr) 1590, 1565, 1465, 1400, 1260, 1205, 1125, 1040, 895, 755, 700 cm^{-1} .

Preparation of 3-phenyl-1(3H)-isobenzo[c]thiophene-1-thione6.5⁷⁹

2-Benzoylbenzoic acid (6.0g, 26mmol.) and Lawesson's reagent (21.46g, 52mmol.) were dissolved in toluene (10mL) and refluxed for 8 hours followed by stirring for 2 days. The toluene was removed under reduced pressure and the residue was purified by dry flash chromatography followed by radial silica chromatography to give 3-phenyl-1(3H)-isobenzo[c]thiophene-1-thione as chunky orange crystals (2.5g, 40%).

m.p. 93-94°C (lit. 93-94°C).

^1H NMR (CDCl_3) δ 5.97 (s, 1H, SCH), 7.21-7.28 (m, 3H, aryl CH), 7.30-7.38 (m, 3H, aryl CH), 7.49 (m, 1H, aryl CH), 7.59 (m, 1H, aryl CH), 8.15 (d, 1H, $J=7.6\text{Hz}$, aryl CH).

^{13}C NMR (CDCl_3) δ 60.6, 124.5, 126.0, 128.3, 128.5, 128.6, 129.2, 133.0, 137.5, 143.6, 151.2, 227.2.

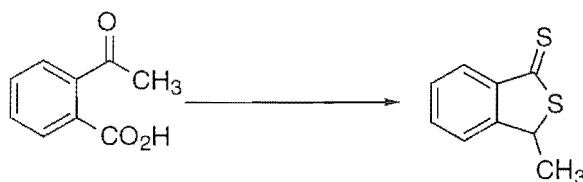
m/z (EI) 242.0 (M^+), 210.1 (M-S^+), 165.1 (M-SH_2^+), 121.0.

High Res. m/z $\text{C}_{14}\text{H}_{10}\text{S}_2$ calc. 242.02220; found 242.02240.

IR (KBr) ν_{max} 3071, 3032, 2239, 1690, 1578, 1497, 1454, 1269, 1244, 1217, 1182, 1049, 903, 745, 712 cm^{-1} .

Preparation of 3-methyl-1(3H)-isobenzo[c]thiophene-1-thione

6.779



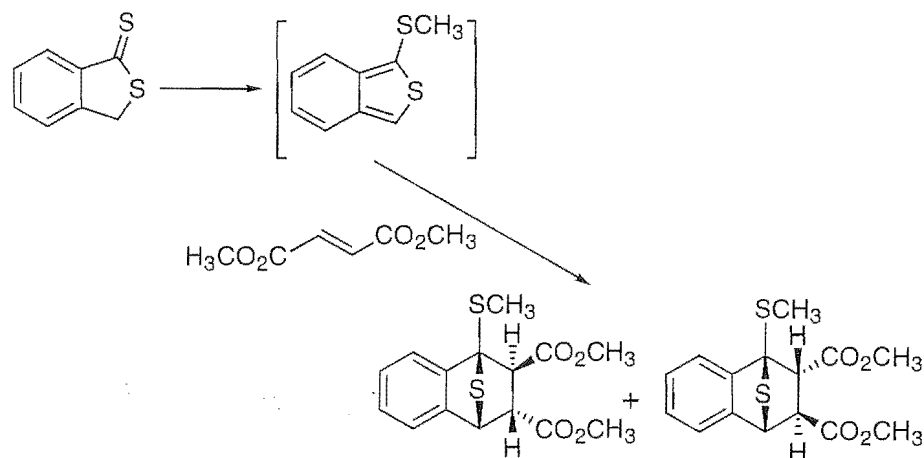
A solution of 2-acetylbenzoic acid (2.00g, 12.2mmol.) and Lawesson's reagent (4.93g, 12.2mmol.) in toluene (10mL) was refluxed under nitrogen for 45 minutes. The toluene was removed under reduced pressure and the residue dissolved in ether and filtered to remove ether insoluble components. The ether was removed under reduced pressure and purification by radial silica chromatography gave 3-methyl-1(3H)-isobenzo[c]thiophene-1-thione as a red oil (198mg, 9%).

^1H NMR (CDCl_3) δ 1.78 (d, 3H, $J=6.8\text{Hz}$, CH_3), 4.93 (q, 1H, $J=6.8\text{Hz}$, CH), 7.44-7.70 (m, 3H, aryl CH), 8.04 (d, 1H, $J=7.8\text{Hz}$, aryl CH).

^{13}C NMR (CDCl_3) δ 20.3, 51.1, 124.1, 128.0, 132.6, 143.1, 151.9, 227.4.

IR ν_{max} (CDCl_3) 1600, 1465, 1260, 1050, 760, 740 cm^{-1} .

Preparation of dimethyl 9-*exo*-10-*endo*-methylsulfanyl-11-thiatricyclo[6.2.1.0^{2,7}]undeca-2,4,6-triene-9,10-dicarboxylate **6.9 and dimethyl 9-*endo*-10-*exo*-methylsulfanyl-11-thiatricyclo[6.2.1.0^{2,7}]undeca-2,4,6-triene-9,10-dicarboxylate **6.10****



n-Butyl lithium (0.70mL of a 1.0M solution in hexane, 1.1mmol.) was added dropwise to a stirred solution of diisopropylamine (0.16mL, 1.1mmol.) in THF (1mL) at 0°C. The solution was stirred for ten minutes at 0°C and then cooled to -78°C. A solution of 1,3-dihydro-2-benzothiophene-1-thione (170mg, 1.0mmol.) in THF (1mL) was added and the mixture was stirred for 1 hour at -78°C. Methyl iodide (0.07mL, 1.1mmol.) was added dropwise to the solution, which was then allowed to warm to room temperature for 2 hours. Dimethyl fumarate (162mg, 1.1mmol.) in THF (1mL) was added and the reaction stirred overnight. The solvent was removed under reduced pressure to give an oil which was partitioned between ethyl acetate and water. The organic layer was dried (MgSO₄) and the solvent removed under reduced pressure. This was purified by radial silica chromatography to give a mixture of dimethyl 9-*exo*-10-*endo*-methylsulfanyl-11-thiatricyclo[6.2.1.0^{2,7}]undeca-2,4,6-triene-9,10-dicarboxylate **6.9** and dimethyl 9-*endo*-10-*exo*-methylsulfanyl-11-thiatricyclo[6.2.1.0^{2,7}]undeca-2,4,6-triene-9,10-dicarboxylate **6.10** together as a pale yellow oil (30mg, 9%).

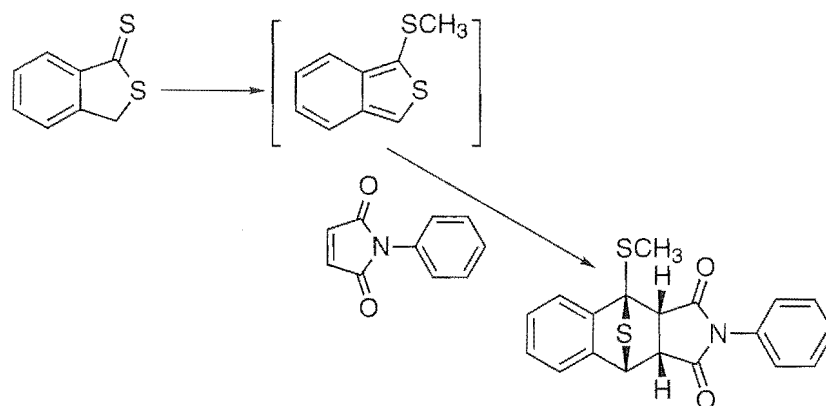
6.9 (major) ^1H NMR (CDCl_3) δ 2.30 (s, 3H, SCH₃), 3.33 (d, 1H, $J=4.4\text{Hz}$, COCH), 3.58 (s, 3H, CO₂CH₃), 3.83 (s, 3H, CO₂CH₃), 4.21 (d, 1H, $J=4.4\text{Hz}$, COCH), 4.42 (m, 1H, SCH), 7.07-7.34 (m, 4H, aryl CH).

6.10 (minor) δ 2.19 (s, 3H, SCH₃), 3.61 (d, 1H, $J=4.4\text{Hz}$, COCH), 3.55 (s, 3H, CO₂CH₃), 3.81 (s, 3H, CO₂CH₃), 4.81 (d, 1H, $J=4.4\text{Hz}$, COCH), 4.92 (m, 1H, SCH), 7.07-7.34 (m, 4H, aryl CH).

m/z (EI) 324 (M^+), 180 ($\text{M}-\text{C}_6\text{H}_6\text{O}_4^+$).

High Res. $\text{C}_{15}\text{H}_{16}\text{S}_2\text{O}_4$ m/z calc. 324.04906; found 324.04915.

Preparation of 9-endo-13-endo-1-methylsulfanyl-11-phenyl-11-aza-14-thiatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione **6.11**



n-Butyl lithium (0.041mL of a 1.0M solution in hexane, 0.066mmol.) was added dropwise to a stirred solution of diisopropylamine (0.0093mL, 0.066mmol.) in THF at 0°C. The solution was stirred for ten minutes at 0°C and then cooled to -78°C. A solution of 1(3H)-isobenzocyclopentathien-1-thione (11mg, 0.066mmol.) in THF was added and the mixture was stirred for 1 hour at -78°C. Methyl iodide (0.0045mL, 0.073mmol.) was added dropwise to the solution, which was then allowed to warm to room temperature for 1.5 hours. *N*-Phenyl maleimide (12.5mg, 0.073mmol.) in THF was added and the reaction stirred overnight. The

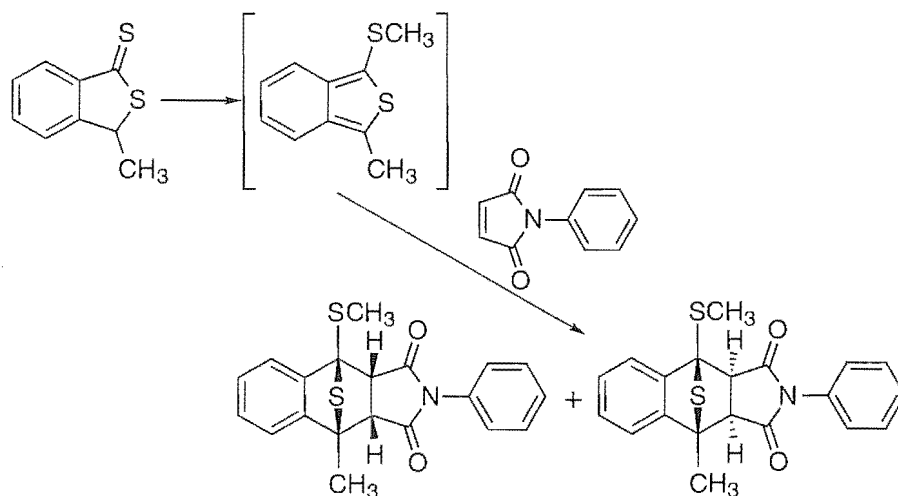
solvent was removed under reduced pressure to give an oil which was partitioned between ethyl acetate and water. The organic layer was dried (MgSO_4) and the solvent removed under reduced pressure. This was purified by radial silica chromatography to give 9-*endo*-13-*endo*-1-methylsulfanyl-11-phenyl-11-aza-14-thiatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione as a brown oil (1.5mg, 6%).

^1H NMR (CDCl_3) δ 2.25 (s, 3H, SCH_3), 3.25 (d, 1H, $J=6.8\text{Hz}$, COCH), 3.57 (d, 1H, $J=6.8\text{Hz}$, COCH), 4.97 (s, 1H, SCH), 7.08-7.48 (m, 9H, aryl CH).

m/z (EI/ FAB) 353 (M^+), 180, 173.

High Res. $\text{C}_{19}\text{H}_{15}\text{NS}_2\text{O}_2$ m/z calc. 353.05448; found 353.05500.

Preparation of 9-*endo*-13-*endo*-1-methylsulfanyl-8-methyl-11-phenyl-11-aza-14-thiatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione 6.13 and 9-*exo*-13-*exo*-1-methylsulfanyl-8-methyl-11-phenyl-11-aza-14-thiatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione 6.14



n-Butyl lithium (0.14mL of a 1.0M solution in hexane, 0.22mmol.) was added dropwise to a stirred solution of diisopropylamine (0.031mL, 0.22mmol.) in THF at 0°C. The solution was stirred for ten minutes at 0°C and then cooled to -78°C. A

solution of 3-methyl-1,3-dihydro-2-benzothiophene-1-thione (37.9mg, 0.22mmol.) in THF was added and the mixture was stirred for 1 hour at -78°C. Methyl iodide (0.014mL, 0.22mmol.) was added dropwise to the solution, which was then allowed to warm to room temperature for 2 hours. *N*-Phenyl maleimide (38.4mg, 0.22mmol.) in THF was added and the reaction stirred overnight. The solvent was removed under reduced pressure to give an oil which was partitioned between ethyl acetate and water. The organic layer was dried (MgSO₄) and the solvent removed under reduced pressure. This was purified by radial silica chromatography to give both 9-*endo*-13-*endo*-1-methylsulfanyl-8-methyl-11-phenyl-11-aza-14-thiatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione **6.14** and 9-*exo*-13-*exo*-1-methylsulfanyl-8-methyl-11-phenyl-11-aza-14-thiatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione **6.13** together as a yellow oil (6mg, 7%).

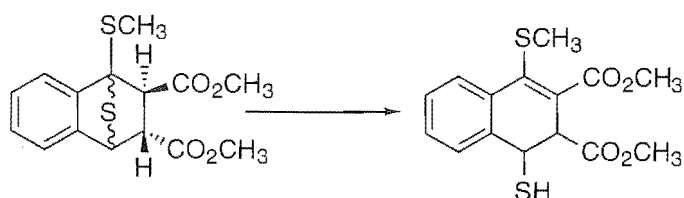
6.14 (major) ¹H NMR (CDCl₃) δ 2.16 (s, 3H, CH₃), 2.32 (s, 3H, SCH₃), 4.07 (d, 1H, J=8.3Hz, COCH), 4.23 (d, 1H, J=8.3Hz, COCH), 6.40 (m, 1H, aryl CH), 7.16-7.47 (m, 8H, aryl CH).

6.13 (minor) δ 2.13 (s, 3H, CH₃), 2.24 (s, 3H, SCH₃), 3.21 (d, 1H, J=6.3Hz, COCH), 3.34 (d, 1H, J=6.3Hz, COCH), 6.40 (m, 1H, aryl CH), 7.16-7.47 (m, 8H, aryl CH).

m/z (EI) 367 (M⁺), 333 (M-SH₂⁺), 194, 173.

High Res. C₂₀H₁₇NS₂O₂ *m/z* calc. 367.07008; found 367.06980.

Preparation of dimethyl 1-mercapto-4-methylsulfanyl-1,2-dihydronaphthalene-2,3-dicarboxylate 6.18

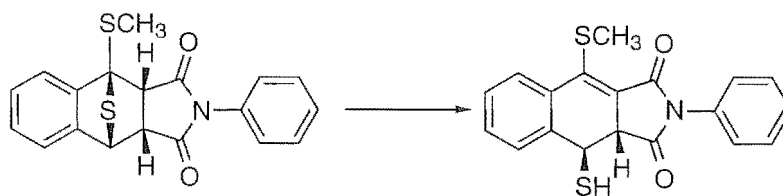


Dimethyl methylsulfanyl-11-thiatricyclo[6.2.1.0^{2,7}]undeca-2,4,6-triene-9,10-dicarboxylate (10mg, 0.03mmol.) and mercuric acetate (9.6mg, 0.03mmol.) were dissolved in methanol (1mL) and stirred for 5 days. The methanol was removed under reduced pressure and the product partitioned between dichloromethane and water. The dichloromethane fractions were combined, dried (MgSO₄), and the dichloromethane removed under reduced pressure. The product was purified by radial silica chromatography to give dimethyl 1-mercapto-4-methylsulfanyl-1,2-dihydronaphthalene-2,3-dicarboxylate as a yellow oil (2.6mg, 26%).

¹H NMR (CDCl₃) δ 2.21 (s, 3H, SCH₃), 3.56 (s, 3H, CO₂CH₃), 3.88 (s, 3H, CO₂CH₃), 4.30 (d, 1H, J=6.0Hz, COCH), 5.08 (d, 1H, J=6.0Hz, C(SH)H), 7.30-7.60 (m, 3H, aryl CH), 7.74 (d, 1H, J=7.3Hz, aryl CH).

m/z (EI) 324 (M⁺), 306 (M-H₂O⁺), 180 (M-C₆H₆O₄⁺).

Preparation of *N*-phenyl 1-mercapto-4-methylsulfanyl-1,2-dihydronaphthalene-2,3-dicarboximide 6.19



9-*endo*-13-*endo*-1-Methylsulfanyl-11-phenyl-11-aza-14-thiatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione (1.0mg, 0.0028mmol.) and

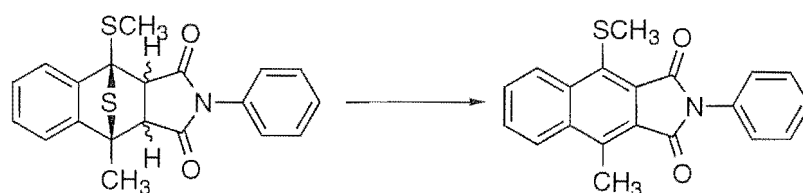
mercuric acetate (0.9mg, 0.0028mmol.) were dissolved in methanol (1mL) and stirred for 5 days. The methanol was removed under reduced pressure and the product partitioned between dichloromethane and water. The dichloromethane fractions were combined, dried (MgSO₄), and the dichloromethane removed under reduced pressure. The product was purified by radial silica chromatography to give *N*-phenyl 1-mercapto-4-methylsulfanyl-1,2-dihydronaphthalene-2,3-dicarboximide as an oil (0.4mg, 40%).

¹H NMR (CDCl₃) δ 2.41 (s, 3H, SCH₃), 3.90 (s, 1H, C(SH)H), 7.64-7.81 (m, 5H, aryl CH), 7.98 (m, 1H, aryl CH), 8.07 (d, 1H, J=5.9Hz, aryl CH), 8.70 (d, 1H, J=7.8Hz, aryl CH), 8.99 (d, 1H, J=7.8Hz, aryl CH).

m/z (EI/ FAB) 353 (M⁺), 317 (M-SH₂⁺).

High Res. C₁₉H₁₅NS₂O₂ *m/z* calc. 353.05448; found 353.05398.

Preparation of *N*-phenyl 1-methylsulfanyl-8-methyl-naphthalene-2,3-dicarboximide 4.33

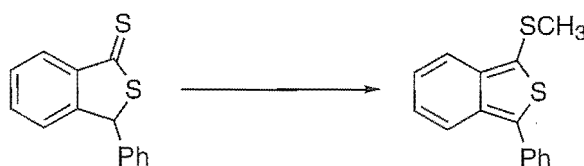


1-methylsulfanyl-8-methyl-11-phenyl-11-aza-14-thiatetracyclo [6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione (4mg, 0.01mmol.) and mercuric acetate (3.2mg, 0.01mmol.) were dissolved in methanol (1mL) and stirred for 1 week. The methanol was removed under reduced pressure to give an oil which was purified by radial silica chromatography to give *N*-phenyl 1-methylsulfanyl-8-methyl-naphthalene-2,3-dicarboximide as a white solid (2.8mg, 84%).

^1H NMR (CDCl_3) δ 2.62 (s, 3H, CH_3), 3.17 (s, 3H, SCH_3), 7.43-7.56 (m, 5H, aryl CH), 7.80 (m, 2H, aryl CH), 8.30 (m, 1H, aryl CH), 9.05 (m, 1H aryl CH).

Preparation of 1-methylsulfanyl-3-phenyl-isobenzo[c]thiophene

6.20⁸⁰



n-Butyl lithium (6.16mL of a 1.6M solution in hexane, 10mmol.) was added dropwise to a stirred solution of diisopropylamine (0.92mL, 10mmol.) in THF at 0°C under dry nitrogen. The solution was stirred for ten minutes at 0°C and then cooled to -78°C. A solution of 3-phenyl-1(3H)-isobenzo[c]thiophene-1-thione (1.0g, 4mmol.) in THF was added and the mixture was stirred for 1 hour at -78°C. Methyl iodide (0.66mL, 10mmol.) was added dropwise to the solution, which was then allowed to warm to room temperature over a period of 2 hours. The THF was removed under reduced pressure and the product purified by radial silica chromatography to give 1-methylsulfanyl-3-phenyl-isobenzo[c]thiophene as a yellow oil (0.9g, 88%).

^1H NMR (CDCl_3) δ 2.51 (s, 3H, SCH_3), 7.10 (m, 1H, aryl CH), 7.17 (m, 1H, aryl CH), 7.38 (m, 1H, aryl CH), 7.48 (m, 2H, aryl CH), 7.50 (m, 2H, aryl CH), 7.80 (m, 2H, aryl CH).

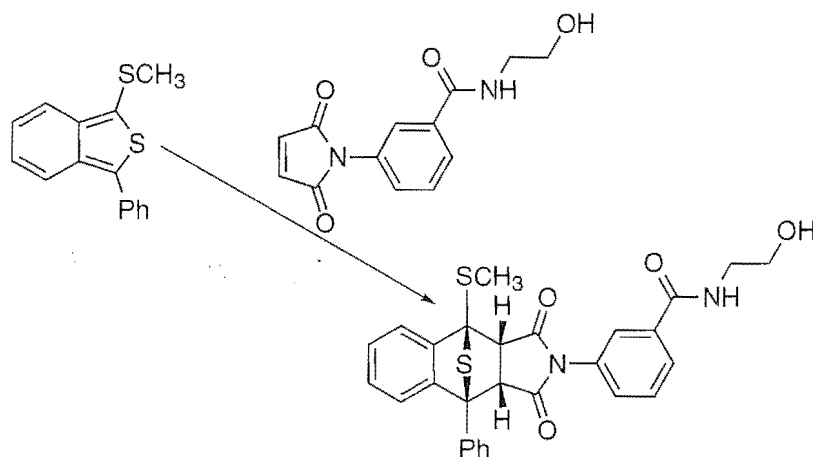
^{13}C NMR (CDCl_3) δ 22.4, 118.0, 120.2, 120.6, 123.7, 123.8, 127.2, 128.5, 128.7, 133.4, 124.1, 144.2.

m/z (EI) 256.0 (M^+), 241.0, ($\text{M}-\text{CH}_3^+$), 208.0, 143.0.

High Res. m/z $\text{C}_{15}\text{H}_{12}\text{S}_2$ calc. 256.03784; found 256.03805.

IR (KBr) ν_{\max} 3693.4, 3064.7, 2923.9, 1600.8, 1504.4, 1485.1, 1444.6, 1365.5, 1334.6, 1313.4, 1203.5, 1078.1 cm^{-1} .

Preparation of 9-endo-13-endo-1-methylsulfanyl-8-phenyl-11-(3-(2-aza-4-hydroxy-1-oxobutanyl)phenyl)-11-aza-14-thiatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione 6.21



1-Methylsulfanyl-3-phenyl-isobenzo[c]thiophene (64mg, 0.25mmol.) and *N*-(3-(2-aza-4-hydroxy-1-oxobutanyl)phenyl) maleimide (65mg, 0.25mmol.) were dissolved in toluene (10mL) and refluxed at 120°C for 48 hours. The toluene was removed under reduced pressure and the resulting oil purified by radial silica chromatography to give a solid. Recrystallisation from ethyl acetate and pet ether gave 9-endo-13-endo-1-methylsulfanyl-8-phenyl-11-(3-(2-aza-4-hydroxy-1-oxobutanyl)phenyl)-11-aza-14-thiatetracyclo[6.5.1.0^{2,7}.0^{9,13}] tetradeca-2,4,6-triene-10,12-dione as white crystals (9mg, 7%).

m.p. 167-168°C.

Analysis $\text{C}_{28}\text{H}_{24}\text{N}_2\text{S}_2\text{O}_4$ calc. C, 65.09; H, 4.68; N, 5.42; found C, 65.24, H, 4.28, N, 5.61.

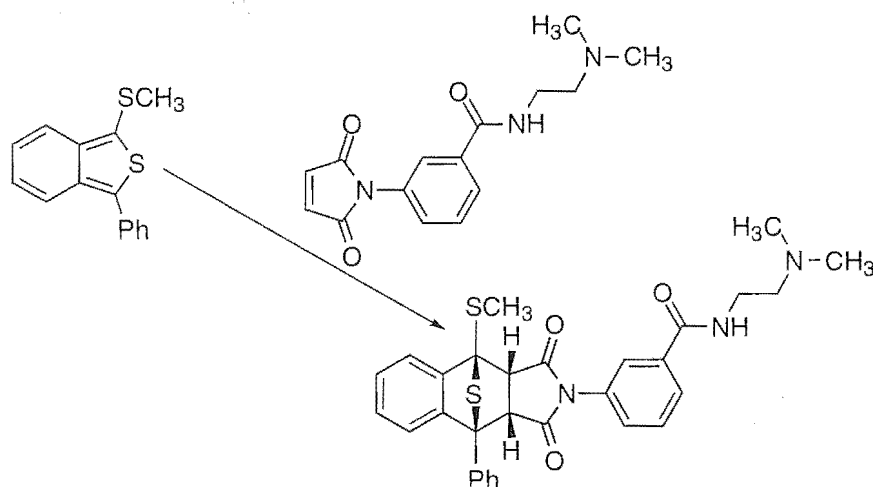
^1H NMR (CDCl_3) δ 2.41 (s, 3H, SCH_3), 3.54 (m, 2H, NHCH_2), 3.73 (m, 2H, CH_2OH), 4.45 (d, 1H, $J=8.3\text{Hz}$, COCH), 4.98 (d, 1H, $J=8.3\text{Hz}$, COCH),

6.55 (d, 1H, $J=7.3\text{Hz}$, aryl CH), 6.83 (d, 1H, $J=7.3\text{Hz}$, aryl CH), 6.92 (s, 1H, aryl CH), 7.21 (d, 1H, $J=7.8\text{Hz}$, aryl CH), 7.31-7.51 (m, 6H, aryl CH), 7.72 (d, 1H, $J=7.8\text{Hz}$, aryl CH), 7.88 (d, 2H, $J=7.8\text{Hz}$, aryl CH).

m/z (EI) 516 (M^+), 498 ($M-H_2O^+$), 260, 256.

High Res. $C_{28}H_{24}N_2S_2O_4$ m/z calc. 516.11784; found 516.11810.

Preparation of 9-endo-13-endo-1-methylsulfanyl-8-phenyl-11-(3-(2-aza-4-dimethylamino-1-oxobutanyl)phenyl)-11-aza-14-thiatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione
6.22



1-Methylsulfanyl-3-phenyl-isobenzo[c]thiophene (25mg, 0.10mmol.) and *N*-(3-(2-aza-4-dimethylamino-1-oxobutanyl)phenyl) maleimide (28mg, 0.10mmol.) were dissolved in toluene (10mL) and refluxed at 120°C for 4 days. The toluene was removed under reduced pressure and the resulting oil purified by radial silica chromatography to give 9-endo-13-endo-1-methylsulfanyl-8-phenyl-11-(3-(2-aza-4-dimethylamino-1-oxobutanyl)phenyl)-11-aza-14-thiatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione as an oil (2.0mg, 4%).

^1H NMR ($\text{CDCl}_3/\text{CD}_3\text{OD}$) δ 2.32 (s, 3H, SCH_3), 2.62 (s, 6H, $\text{N}(\text{CH}_3)_2$), 3.20 (m, 2H, CH_2), 3.78 (m, 2H, CH_2), 4.36 (d, 1H, $J=8.3\text{Hz}$, COCH), 4.88

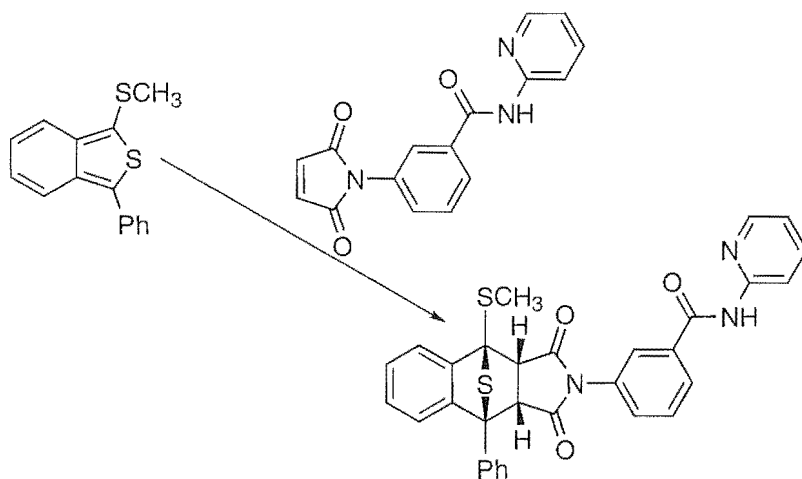
(d, 1H, $J=8.3\text{Hz}$, COCH), 6.35 (d, 1H, $J=8.8\text{Hz}$, aryl CH), 6.74 (d, 1H, $J=7.4\text{Hz}$, aryl CH), 7.10-7.29 (m, 3H, aryl CH), 7.31-7.44 (m, 4H, aryl CH), 7.71 (d, 1H, $J=8.3\text{Hz}$, aryl CH), 7.82 (d, 2H, $J=8.3\text{Hz}$, aryl CH), 8.46 (br.s, 1H, NH).

m/z (EI) 543 (M^+), 429 ($M-C_5H_{10}N_2O^+$), 256, 241.

High Res. $C_{30}H_{29}N_3S_2O_3$ m/z calc. 543.16515; found 543.16549.

IR (CDCl₃/ CD₃OD) ν_{max} 2970.2, 1716.5, 1660.6, 1602.7, 1456.2, 1379.0, 1236.3, 1188.1 cm^{-1} .

Preparation of 9-endo-13-endo-1-methylsulfanyl-8-phenyl-11-(3-(*N*-(2-pyridyl))carboxamidophenyl)-11-aza-14-thiatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione 6.23



1-Methylsulfanyl-3-phenyl-isobenzo[c]thiophene (52mg, 0.20mmol.) and *N*-3-(*N*-(2-pyridyl))carboxamidophenyl maleimide (66mg, 0.20mmol.) were dissolved in toluene (10mL) and refluxed at 120°C for 3 days. The toluene was removed under reduced pressure and the resulting oil purified by radial silica chromatography to give 9-endo-13-endo-1-methylsulfanyl-8-phenyl-11-(3-(*N*-(2-pyridyl))carboxamidophenyl)-11-aza-14-thiatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione as an oil (4.4mg, 4%).

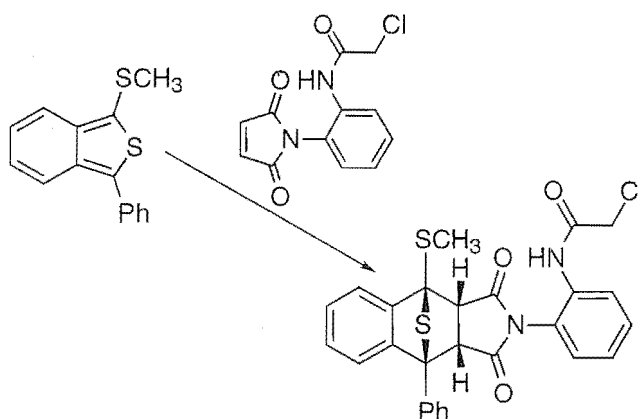
^1H NMR ($\text{CDCl}_3/\text{CD}_3\text{OD}$) δ 2.27 (s, 3H, SCH_3), 4.35 (d, 1H, $J=8.3\text{Hz}$, COCH), 4.89 (d, 1H, $J=8.3\text{Hz}$, COCH), 5.60 (d, 1H, $J=8.3\text{Hz}$, aryl CH), 6.72 (d, 1H, $J=7.3\text{Hz}$, aryl CH), 6.89 (t, 1H, $J=7.3\text{Hz}$, aryl CH), 7.10 (t, 1H, $J=7.3\text{Hz}$, aryl CH), 7.18 (m, 3H, aryl CH), 7.27-7.38 (m, 6H, aryl CH), 7.55 (d, 1H, $J=7.8\text{Hz}$, aryl CH), 7.61 (m, 1H, aryl CH), 7.74 (d, 2H, $J=7.3\text{Hz}$, aryl CH).

m/z (FAB) 550.1 (M^+), 460.1.

High Res. $\text{C}_{31}\text{H}_{23}\text{N}_3\text{S}_2\text{O}_3$ m/z calc. 550.12591; found 550.12575.

IR ($\text{CDCl}_3/\text{CD}_3\text{OD}$) ν_{max} 1716.5, 1600.8, 1458.1, 1377.1, 1236.3 cm^{-1} .

Preparation of 9-endo-13-endo-1-methylsulfanyl-8-phenyl-11-(2-chloroacetamidophenyl)-11-aza-14-thiatetracyclo[6.5.1.0^{2,7}.0^{9,13}] tetradeca-2,4,6-triene-10,12-dione 6.25



1-Methylsulfanyl-3-phenyl-isobenzo[*c*]thiophene (51.6mg, 0.2mmol.) and *N*-(2-chloroacetamidophenyl) maleimide (50mg, 0.2mmol.) were dissolved in toluene (10mL) and refluxed at 120°C for 48 hours. The toluene was removed under reduced pressure and the resulting oil purified by radial silica chromatography to give 9-endo-13-endo-1-methylsulfanyl-8-phenyl-11-(2-chloroacetamidophenyl)-11-aza-14-thiatetracyclo[6.5.1.0^{2,7}.0^{9,13}] tetradeca-2,4,6-triene-10,12-dione as white crystals (8mg, 8%).

m.p. 252-254°C.

Analysis $C_{27}H_{21}N_2S_2O_3Cl$ calc. C, 62.24; H, 4.06; N, 5.38; found C, 62.20, H, 3.97; N, 5.40.

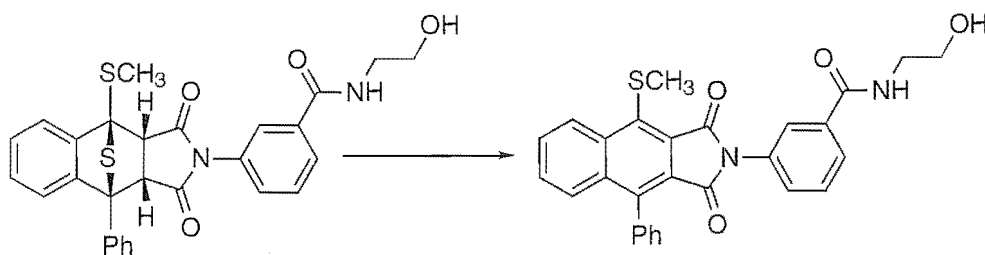
1H NMR ($CDCl_3$) δ 2.38 (s, 3H, SCH₃), 4.13 (s, 2H, CH₂Cl), 4.45 (d, 1H, $J=8.3$ Hz, COCH), 4.96 (d, 1H, $J=8.3$ Hz, COCH), 5.75 (d, 1H, $J=7.8$ Hz, aryl CH), 6.83 (d, 1H, $J=8.3$ Hz, aryl CH), 6.99 (t, 1H, $J=7.8$ Hz, aryl CH), 7.17-7.32 (m, 3H, aryl CH), 7.39-7.49 (m, 4H, aryl CH), 7.71 (d, 1H, $J=8.3$ Hz, aryl CH), 7.85 (d, 2H, $J=8.3$ Hz, aryl CH), 8.17 (br.s, 1H, NH).

m/z (EI) 520 (M^+), 485 ($M-Cl^+$), 444, 256.

High Res. $C_{27}H_{21}N_2S_2O_3Cl$ m/z calc. 520.06829; found 520.0649.

IR ($CDCl_3$) ν_{max} 1716.5, 1521.7, 1458.1, 1375.2, 1186.1, 1157.2 cm^{-1} .

Preparation of *N*-3-(2-aza-4-hydroxy-1-oxobutanyl)phenyl 1-methylsulfanyl-8-phenyl-naphthalene-2,3-dicarboximide 5.27

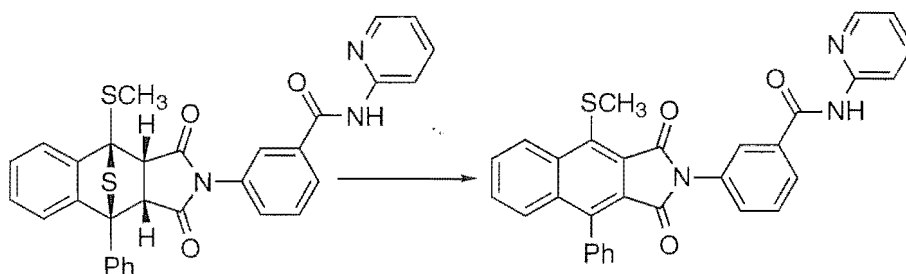


9-*endo*-13-*endo*-1-Methylsulfanyl-8-phenyl-11-(3-(2-aza-4-hydroxy-1-oxobutanyl)phenyl)-11-aza-14-thiatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione (3mg, 0.006mmol.) and mercuric acetate (1.9mg, 0.006mmol.) were dissolved in methanol (1mL) and stirred for 1 week. The methanol was removed under reduced pressure to give an oil which was purified by radial silica chromatography to give *N*-3-(2-aza-4-hydroxy-1-oxobutanyl)phenyl 1-methylsulfanyl-8-phenyl-naphthalene-2,3-dicarboximide as an oil (0.4mg, 14%).

1H NMR ($CDCl_3/CD_3OD$) δ 2.47 (s, 3H, SCH₃), 3.62 (m, 2H, NHCH₂), 4.40 (t, 2H, $J=6.8$ Hz, CH₂OH), 6.93 (d, 1H, $J=7.3$ Hz, aryl CH), 7.09 (m, 4H,

IR (CDCl₃/ CD₃OD) ν_{\max} 2360.7, 2343.4, 1718.5, 1236.3 cm⁻¹.

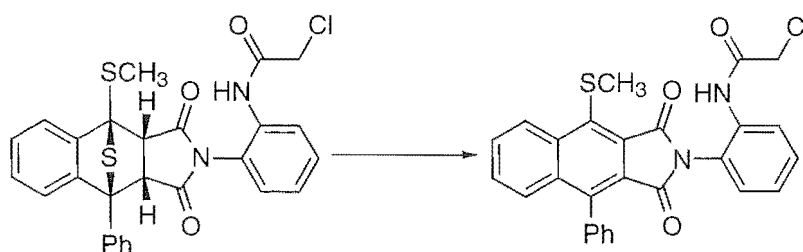
Preparation of *N*-3-(*N*-(2-pyridyl))carboxamidophenyl 1-methylsulfanyl-8-phenyl-naphthalene-2,3-dicarboximide 5.29



9-*endo*-13-*endo*-1-Methylsulfanyl-8-phenyl-11-(3-(*N*-(2-pyridyl))carboxamidophenyl)-11-aza-14-thiatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione (3mg, 0.0055mmol.) and mercuric acetate (1.7mg, 0.0055mmol.) were dissolved in methanol (1mL) and stirred for 1 week. The methanol was removed under reduced pressure to give an oil which was purified by radial silica chromatography to give *N*-3-(*N*-(2-pyridyl))carboxamidophenyl 1-methylsulfanyl-8-phenyl-naphthalene-2,3-dicarboximide as an oil (0.3mg, 11%).

¹H NMR (CDCl₃/ CD₃OD) δ 2.69 (s, 3H, SCH₃), 7.18-7.43 (m, 5H, aryl CH), 7.47-7.82 (m, 8H, aryl CH), 7.91-8.11 (m, 3H, aryl CH), 9.08 (d, 1H, J=8.8Hz, aryl CH).

Preparation of *N*-2-(chloroacetamido)phenyl 1-methylsulfanyl-8-phenyl-naphthalene-2,3-dicarboximide 5.11



9-*endo*-13-*endo*-1-Methylsulfanyl-8-phenyl-11-(2-chloroacetamidophenyl)-11-aza-14-thiatetracyclo[6.5.1.0^{2,7}.0^{9,13}]tetradeca-2,4,6-triene-10,12-dione (3mg, 0.0057mmol.) and mercuric acetate (1.8mg, 0.0057mmol.) were dissolved in methanol (1mL) and stirred for 1 week. The methanol was removed under reduced pressure and the product partitioned between dichloromethane and water. The dichloromethane fractions were combined, dried (MgSO₄), and the dichloromethane removed under reduced pressure. The product was purified by radial silica chromatography to give *N*-2-(chloroacetamido)phenyl 1-methylsulfanyl-8-phenyl-naphthalene-2,3-dicarboximide as white crystals (0.8mg, 29%).

¹H NMR (CDCl₃) δ 2.70 (s, 3H, SCH₃), 3.69 (s, 2H, CH₂Cl), 7.24-7.44 (m, 6H, aryl CH), 7.51 (m, 2H, aryl CH), 7.64 (t, 1H, J=6.8Hz, aryl CH), 7.75-7.83 (m, 2H, aryl CH), 8.06 (m, 1H, aryl CH), 9.07 (d, 1H, J=9.8Hz, aryl CH).

APPENDIX

A1 Biological Testing: The P388 Anti-cancer Assay

Many of the compounds prepared in this thesis were tested for biological activity, in particular anti-cancer activity, via the P388 assay. This assay uses the P388 cell line of murine leukemia cells. It is a cytotoxic based assay against this specific cell type and is up to one hundred times more sensitive to cytotoxic effects than the anti-viral BSC-1 line. The result obtained is in the form of an ID₅₀, which represents the concentration of the test compound at which the number of viable P388 cells is reduced to 50% relative to the control, and is expressed here in micrograms per millilitre ($\mu\text{g/mL}$). Although the P388 cell line represents a rapidly dividing cell type, a compound found active by this assay may also display selective activity against the slow growing tumours and is worthy of further consideration.⁸²

P388 Assay Results

Compound Number	ID ₅₀ (μg/mL)
2.19	51.00
2.33	>25.00
3.15	>62.50
3.22	>62.50
3.23	62.50
3.24	>31.25
3.25	4.39
4.19, 4.20	>62.50
4.26	>62.50
4.27	>25.00
4.28	>25.00
4.29	>62.50
4.32	>12.50
4.33	>14.58
4.34	>20.00
4.44	>62.50
4.45	>62.50
4.48	>62.50
4.49	>17.50
4.50	>27.50
4.51	>26.25
4.52	>12.50
4.53	5.85

Compound Number	ID ₅₀ (μg/mL)
4.54	>26.25
4.55	9.67
4.56	>31.25
4.57	>12.50
4.58	>12.50
4.59	>23.75
4.60	>10.00
4.61	>10.00
4.62	>7.50
4.63	>15.00
4.71	>25.00
4.72	>31.00
5.9	1.26
5.10	0.43
5.11	3.03
5.12	2.53
5.20	2.27
5.21	8.15
5.24	10.99
5.25	4.99
5.27	34.60
5.28	51.00
5.29	>6.25
5.30	8.11

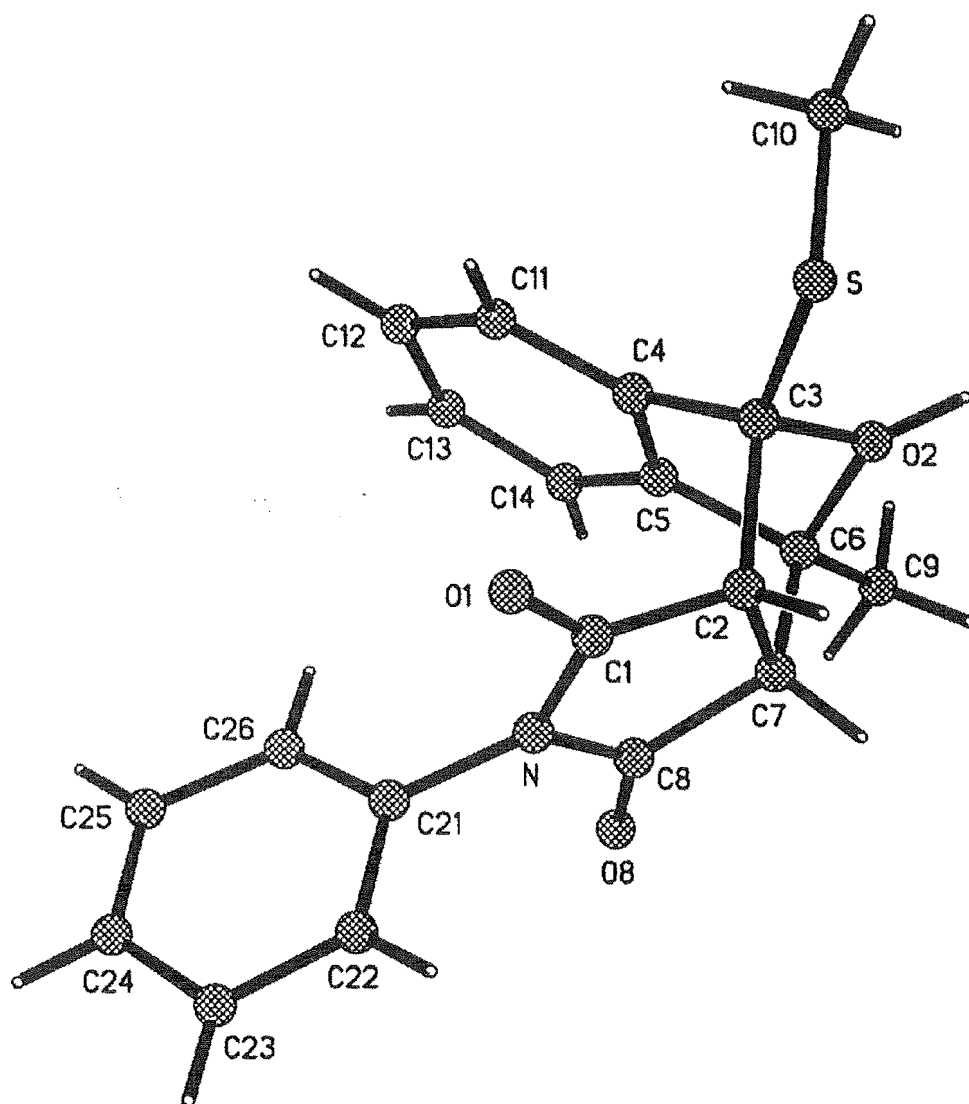
Compound Number	ID ₅₀ (μg/mL)
6.9, 6.10	>62.50
6.11	51.00
6.13, 6.14	>20.00
6.18	>50.00
6.19	51.00
6.21	0.24
6.22	7.98
6.23	2.23
6.25	38.34
6.26	2.86

A2 X-Ray Crystal Structure of 4.27

A2.1 Method of Data Collection and Structure Refinement

A unique data set was measured at 293 (2)K within $2\theta_{\max} = 57^\circ$ limit. Of the 2703 reflections obtained, 2623 were unique ($R_{\text{int}} = 0.1557$) and were used in the full-matrix least-squares refinement [SHELXL-93 (Sheldrick, 1993)] after being corrected for absorption by using the psi-scan method.⁸³ The intensities of 3 standard reflections, measured every 97 reflections throughout the data collection, showed no decay. The structure was solved by direct methods [SHELXL-86 (Sheldrick, 1990)].⁸⁴ Hydrogen atoms were fixed in idealised positions. All non-hydrogen atoms were refined with anisotropic atomic displacement parameters. Neutral scattering factors and anomalous dispersion corrections for non-hydrogen atoms were taken from Ibers and Hamilton.⁸⁵

A2.2 Structure of 4.27



A2.2 9-endo-13-endo-1-Methylsulfanyl-8-methyl-11-phenyl-11-aza-14-oxatetracyclo[6.5.1.0^{2,7}.0^{9,13}] tetradeca-2,4,6-triene-10,12-dione

A2.3 Data Tables

Table 1 Crystal data and structure refinement for **4.27**

<i>Crystal data</i>		
Identification code	jmdrt	
Empirical formula	C ₂₀ H ₁₇ NO ₃ S	
Formula weight	351.41	
Crystal description	needles	
Crystal colour	white	
Crystal size	0.9 x 0.45 x 0.14mm	
Crystal system	monoclinic	
Space group	C2	
Unit cell dimensions	a= 23.451 (14)Å $\alpha = 90^\circ$	
	b= 8.090 (4)Å	$\beta = 116.96(5)^\circ$
	c=21.84 (2)Å	$\gamma = 90^\circ$
Reflns to determine unit cell	25	
F (000)	1472	
Volume	3693 (4) Å ³	
Z	8	
Density (calculated)	1.264 Mg/m ³	
Radiation	MoK α λ = 0.71073Å	
	fine-focus sealed tube, graphite monochromator	
Linear absorption coefficient	0.193 mm ⁻¹	
Temperature of measurement	293(2)	
<i>Data collection</i>		
Diffractometer	Siemens P4	
Temperature of data collection	-115°C	
Diffraction measurement method	ω scans	
Reflections collected	2703	
Independent reflections	2623 (R _{int} = 0.1557)	
Gq range for data collection	2.09 to 22.50°	
Range of <i>h</i> , <i>k</i> and <i>l</i>	0 ≤ <i>h</i> ≤ 25, 0 ≤ <i>k</i> ≤ 8, -23 ≤ <i>l</i> ≤ 20	

Check reflections	3 every 97 reflections
-------------------	------------------------

Refinement

Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2623 / 1 / 457
Method of refining H atoms	Riding hydrogens
Goodness-of-fit on F^2	1.118
Final R indices [$I > 2\sigma(I)$]	$R1 = 0.1129$, $wR2 = 0.2845$
R indices (all data)	$R1 = 0.1943$, $wR2 = 0.3498$
Largest diff. peak and hole	1.518 and $-0.445 \text{ e}\text{\AA}^{-3}$
Extinction method	none

Computer processing

Structure solution SHELXS-86 (Sheldrick, 1990)
Structure refinement SHELXL-93 (Sheldrick, 1993)

Table 2 Atomic coordinates and equivalent isotropic displacement parameters for **4.27**

atom	x	y	z	U(eq)
S	9658(3)	7540(10)	4054(2)	35(2)
N	8351(7)	5260(20)	1849(7)	24(4)
O(2)	10138(6)	5370(20)	3506(7)	30(4)
O(1)	8105(7)	6990(20)	526(7)	36(4)
O(8)	8825(7)	3350(20)	469(7)	39(4)
C(1)	8470(10)	5920(30)	2480(10)	33(6)
C(2)	9040(10)	5270(30)	3020(10)	24(5)
C(3)	9620(10)	6510(30)	3330(10)	26(5)
C(4)	9646(8)	7390(30)	760(10)	28(6)
C(5)	9853(8)	6260(30)	430(10)	21(5)
C(6)	9960(10)	4670(30)	2830(10)	29(5)
C(7)	9290(10)	4020(30)	2680(10)	27(5)
C(8)	8790(10)	4130(30)	1920(10)	24(5)
C(9)	10410(10)	3490(30)	2820(10)	36(6)
C(10)	10340(10)	8740(40)	4310(10)	49(7)
C(11)	9430(10)	8990(30)	2470(10)	25(5)
C(12)	9470(10)	9480(30)	1890(10)	28(5)
C(13)	9720(10)	8260(40)	1590(10)	44(7)
C(14)	9880(10)	6690(40)	1840(10)	40(7)
C(21)	7830(10)	5620(30)	1200(10)	35(6)
C(22)	7230(10)	4950(30)	1060(10)	34(6)
C(23)	6720(10)	5230(30)	440(10)	8(5)
C(24)	6820(10)	6280(30)	-40(10)	4(7)
C(25)	7390(10)	6980(40)	100(10)	1(7)
C(26)	7890(10)	6590(30)	720(10)	0(6)

U(eq) is defined as 1/3 the trace of the U_{ij} tensor.

Table 3 Bond lengths (Å) and angles (deg) for **4.27**

S-C(10)	1.73(2)	S-C(3)	1.75(2)
N-C(8)	1.34(3)	N-C(1)	1.38(3)
N-C(21)	1.42(3)	O(2)-C(3)	1.44(3)
O(2)-C(6)	1.45(2)	O(1)-C(1)	1.25(3)
O(8)-C(8)	1.21(2)	C(1)-C(2)	1.42(3)
C(2)-C(7)	1.52(3)	C(2)-C(3)	1.58(3)
C(3)-C(4)	1.46(3)	C(4)-C(5)	1.39(3)
C(4)-C(11)	1.42(3)	C(5)-C(14)	1.35(3)
C(5)-C(6)	1.52(3)	C(6)-C(9)	1.44(3)
C(6)-C(7)	1.53(3)	C(7)-C(8)	1.53(3)
C(11)-C(12)	1.40(3)	C(12)-C(13)	1.44(3)
C(13)-C(14)	1.37(4)	C(21)-C(26)	1.36(3)
C(21)-C(22)	1.42(3)	C(22)-C(23)	1.36(3)
C(23)-C(24)	1.44(3)	C(24)-C(25)	1.36(3)
C(25)-C(26)	1.37(3)		
C(10)-S-C(3)	103(1)	C(8)-N-C(1)	110(2)
C(8)-N-C(21)	122(2)	C(1)-N-C(21)	128(2)
C(3)-O(2)-C(6)	99(1)	O(1)-C(1)-N	121(2)
O(1)-C(1)-C(2)	127(2)	N-C(1)-C(2)	112(2)
C(1)-C(2)-C(7)	106(2)	C(1)-C(2)-C(3)	115(2)
C(7)-C(2)-C(3)	100(2)	O(2)-C(3)-C(4)	100(2)
O(2)-C(3)-C(2)	100(2)	C(4)-C(3)-C(2)	108(2)
O(2)-C(3)-S	112(1)	C(4)-C(3)-S	122(2)
C(2)-C(3)-S	112(1)	C(5)-C(4)-C(11)	120(2)
C(5)-C(4)-C(3)	107(2)	C(11)-C(4)-C(3)	132(2)
C(14)-C(5)-C(4)	120(2)	C(14)-C(5)-C(6)	135(2)
C(4)-C(5)-C(6)	105(2)	C(9)-C(6)-O(2)	113(2)
C(9)-C(6)-C(5)	118(2)	O(2)-C(6)-C(5)	99(2)
C(9)-C(6)-C(7)	118(2)	O(2)-C(6)-C(7)	99(1)
C(5)-C(6)-C(7)	107(2)	C(2)-C(7)-C(8)	102(2)
C(2)-C(7)-C(6)	105(2)	C(8)-C(7)-C(6)	124(2)
N-C(8)-C(7)	110(2)	C(12)-C(11)-C(4)	120(2)
C(11)-C(12)-C(13)	115(2)	C(14)-C(13)-C(12)	124(2)
C(5)-C(14)-C(13)	120(3)	C(26)-C(21)-C(22)	120(2)
C(26)-C(21)-N	123(2)	C(22)-C(21)-N	117(2)
C(23)-C(22)-C(21)	119(2)	C(22)-C(23)-C(24)	118(2)
C(25)-C(24)-C(23)	124(2)	C(24)-C(25)-C(26)	116(2)
C(21)-C(26)-C(25)	124(2)	C(3')-S'-C(10')	104(1)

Table 4 Anisotropic displacement parameters for **4.27**

atom	U11	U22	U33	U23	U13	U12
S	39(3)	42(4)	24(3)	-7(3)	15(2)	2(3)
N	13(8)	40(10)	22(9)	-14(9)	8(7)	1(9)
O(2)	28(8)	40(10)	25(7)	10(8)	10(6)	7(8)
O(1)	29(8)	50(10)	30(8)	4(9)	28(8)	-20(9)
C(1)	20(10)	40(20)	40(10)	10(10)	20(10)	10(10)
C(2)	30(10)	20(10)	30(10)	-10(10)	20(10)	0(10)
C(3)	20(10)	40(20)	4(9)	0(10)	0(8)	10(10)
C(4)	3(9)	30(20)	50(10)	-10(10)	12(9)	-20(10)
C(5)	10(10)	10(10)	40(10)	-10(10)	3(9)	-9(9)
C(6)	40(10)	30(10)	30(10)	10(10)	30(10)	0(10)
C(7)	40(10)	30(10)	10(10)	0(10)	10(10)	-10(10)
C(8)	50(10)	20(10)	0(10)	20(10)	10(10)	-10(10)
C(9)	40(10)	20(10)	50(10)	-20(10)	30(10)	-10(10)
C(10)	70(20)	50(20)	40(10)	-10(10)	40(10)	-10(20)
C(11)	40(10)	20(10)	20(10)	-10(10)	10(10)	0(10)
C(12)	50(10)	10(10)	40(10)	-10(10)	30(10)	-10(10)
C(13)	60(20)	40(20)	40(10)	10(10)	40(10)	-10(20)
C(14)	40(20)	50(20)	40(10)	-10(10)	30(10)	-10(10)
C(21)	40(10)	30(20)	40(10)	10(10)	20(10)	20(10)
C(22)	20(10)	50(20)	30(10)	20(10)	10(10)	0(10)
C(23)	30(10)	10(10)	30(10)	0(10)	10(10)	0(10)
C(24)	40(20)	40(20)	30(10)	10(10)	0(10)	20(10)
C(25)	20(10)	60(20)	40(10)	0(10)	20(10)	0(10)
C(26)	40(10)	30(10)	50(20)	10(10)	20(10)	0(10)

The anisotropic displacement factor exponent takes the form
 $2 \pi^2 [h^2 a^{*2} U(11) + \dots + 2 h k a^* b^* U(12)]$

Table 5 Hydrogen coordinates and equivalent isotropic displacement parameters for **4.27**

atom	x	y	z	U(eq)
H(2A)	10487(6)	5150(20)	3927(7)	36
H(2B)	8950(10)	4750(30)	3380(10)	29
H(7A)	310(10)	2910(30)	2860(10)	32
H(9A)	10820(20)	030(50)	2950(70)	54
H(9B)	10260(30)	000(100)	2370(20)	54
H(9C)	10470(50)	620(90)	3140(50)	54
H(10A)	10420(50)	9300(200)	4730(50)	73
H(10B)	10280(30)	9500(100)	3960(40)	73
H(10C)	10700(20)	8040(40)	4390(80)	73
H(11A)	9250(10)	700(30)	2680(10)	30
H(12A)	9350(10)	0540(30)	1700(10)	34
H(13A)	9760(10)	550(40)	1200(10)	53
H(14A)	10010(10)	5930(40)	1610(10)	48
H(22A)	7180(10)	330(30)	1390(10)	41
H(23A)	6320(10)	760(30)	320(10)	33
H(24A)	6470(10)	490(30)	-460(10)	53
H(25A)	7440(10)	690(40)	-210(10)	49
H(26A)	8290(10)	000(30)	820(10)	47
H(10D)	9500(30)	400(200)	5240(70)	100
H(10E)	8980(90)	300(60)	5310(60)	100
H(10F)	8770(70)	700(100)	4770(10)	100
H(12B)	6760(10)	610(40)	3120(10)	48
H(13B)	6050(10)	940(30)	3250(10)	44
H(14B)	6370(10)	210(30)	3670(10)	39
H(22B)	7300(10)	750(30)	1300(10)	51
H(23B)	6570(10)	270(40)	210(10)	61
H(24B)	5710(10)	500(50)	-40(10)	80
H(25B)	5580(10)	710(40)	870(10)	57

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No-one knows this calm
Like the trees
Arching over swollen water,
Like the subtle pools which are
 Your eyes.
See the stones splashed silent,
Waiting for a smile to awaken,
Or gentle laughter to stir
Greenly amongst the leaves above,
But soft rain is the only sound
 Today